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THE EFFECTS OF CONTINUOUS AND INTERMITTENT TRAINING UPON ATP, PC, CPK, AK AND "M" AND "H" LDH IN SKELETAL MUSCLE, HEART AND LIVER OF THE RAT

by

© LUC ALBERT LEGER

A THESIS
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FACULTY OF GRADUATE STUDIES AND RESEARCH

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ABSTRACT

The purpose of the present study was to investigate the effects of sprint and endurance training upon enzymes associated with anaerobic glycolysis and high energy compound metabolism in rat tissue. Thirty rats were randomly assigned to either sedentary, sprint training or endurance training group. Sprint training consisted of 10 x 1 min run/4 min relief at 70m/min, 8% slope, 5 days/week for 6 months and endurance training, 45 min at 31 m/min, 8% slope, 5 days/week for 6 months. Both training regimens resulted in a decreased activity of LDH, M-LDH and CPK in the fast twitch gastrocnemius, plantaris and tibialis anterior muscles without altering AK activity and PC stores. In the heart and slow twitch soleus, LDH, M-LDH, CPK and AK activities as well as PC stores were unchanged as a result of chronic exercise. No LDH, CPK and AK changes were present in the liver. Both training programs reduced the body weight gain and increased the relative weight of the heart and skeletal muscles. All enzymes and metabolites of heart, liver and slow and fast twitch muscles are affected in a similar manner by sprint and endurance training in the laboratory rat. Sprint training in these animals is presently very empirical and requires further investigation.

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TABLE 1 Abbreviations, Symbols and Definitions

ADP	adenosine-5-diphosphate
Aerobic energy	energy metabolized via the citric acid cycle, O_2 being the final electron carrier
AK	adenylate kinase or ATP:AMP phosphotransferase (EC 2.7.4.3) or myokinase
AMP	adenosine-5-monophosphate
Anaerobic energy	energy metabolized without O_2 via the adenylate kinase, the creatine phosphokinase and the lactate dehydrogenase reactions as well as the use of the ATP stores
ATP	adenosine-5-triphosphate
ATPase	ATP phosphohydrolase (EC 3.6.1.3)
Cgr	continuous training group
CPK	creatine phosphokinase or creatine kinase or ATP: creatine phosphotransferase (EC 2.7.3.2)
Fiber types	The muscle fiber type classification used in this study is that of Edington and Edgerton (1976). There are three types of muscle fibers in the rat: fast-twitch glycolytic (FG), fast-twitch high-oxidative glycolytic (FOG) and slow-twitch oxidative (SO). Fast and slow-twitch (FT and ST) are also used to designate muscle fibers without making any inference to their metabolic state. FT and ST are also used to identify muscles predominantly composed of FT and ST fibers.
FG	fast twitch glycolytic or fast twitch white (see also fiber types)
FOG	fast twitch high oxidative glycolytic or fast twitch red (see also fiber types)
FT	fast twitch or type II (see also fiber types)
LDH	lactate dehydrogenase or total LDH activity or L-lactate: NAD oxidoreductase (EC 1.1.1.27)

TABLE 1 (Continued)

LDH_{21}	LDH activity at PA = 21 x 10^{-4} M
LDH_3	LDH activity at PA = 3 x 10^{-4} M
M-LDH	muscle type monomer of LDH or LDH activity due solely to muscle type monomers
NAD	nicotinamide-adenine dinucleotide
NADH	nicotinamide-adenine dinucleotide, reduced
NADP	nicotinamide-adenine dinucleotide phosphate
NADPH	nicotinamide-adenine dinucleotide phosphate, reduced
GM	gastrocnemius medialis
G6P	D-glucose-6-phosphate
G6P-DH	glucose-6-phosphate dehydrogenase or D-glucose-6-phosphate: NADP oxidoreductose (EC 1.1.1.49)
High energy compounds	ATP + PC
H-LDH	heart type monomer of LDH or LDH activity due solely to heart type monomers
HK	hexokinase or ATP: D-hexose-6-phosphotransferase (EC 2.7.1.1)
Igr	intermittent training group
IU	international unit for enzyme activity (= amount of enzyme which convert 1 micromole of substrate per minute under specific conditions, optimal ionic strength of the buffer, optimal pH, wave length)
n	number of rats per group
P	plantaris or probability level
PA	pyruvate (pyruvic acid)

TABLE 1 (Continued)

PC	N-phosphorylcreatine (creatine phosphate or phosphocreatine)
PH	phosphorylase or α-1,4-glucan: orthophosphate glucosyltransferase (EC 2.4.1.1)
PK	pyruvate kinase or ATP: phosphorylase phosphotransferase (EC 2.7.1.40)
PFK	phosphofructokinase or ATP: D-fructose-6-phosphate 1-phosphotransferase (EC 2.7.1.11)
S	soleus
Sgr	sedentary group
SO	slow-twitch oxidative muscle fiber or slow twitch intermediate (see also fiber types)
ST	slow twitch or type I (see also fiber types)
TA	tibialis anterior
$\dot{V}O_2max$	maximal oxygen uptake
W_{abs}	absolute weight
W_{reg}	regressed weight
W_{rel}	relative weight (i.e. organ weight to body weight ratio)

CHAPTER I

STATEMENT OF THE PROBLEM

Introduction

Muscular contraction is a major element of sport performance. Physical training is carried out in order to improve the various mechanisms that control muscular contraction and hence, sport performance. The present study deals with one aspect of muscular performance, namely, anaerobic metabolism and some of its related components: ATP*, phosphorylcreatine (PC), adenylate kinase (AK, E.C. 2.7.4.3), creatinephosphokinase (CPK, E.C. 2.7.3.2) and lactate dehydrogenase (LDH, E.C. 1.1.1.27). (Figure 1)

Muscular contraction depends on the interaction of the myofibrillar proteins: actin, myosin, troponin and tropomyosin. Contraction is initiated by nervous depolarization via the T tubule system which results in the liberation of the Ca^{++} from the sacroplasmic reticulum, which in turn makes it possible for the myofibrillar proteins to react with ATP (Ashley, 1971; Fabiato and Fabiato, 1977; Margreth et al., 1973; Porter and Franzini-Armstrong, 1965).

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* All abbreviations, symbols and definitions used in this study are explicated in Table 1 (p. xiii).

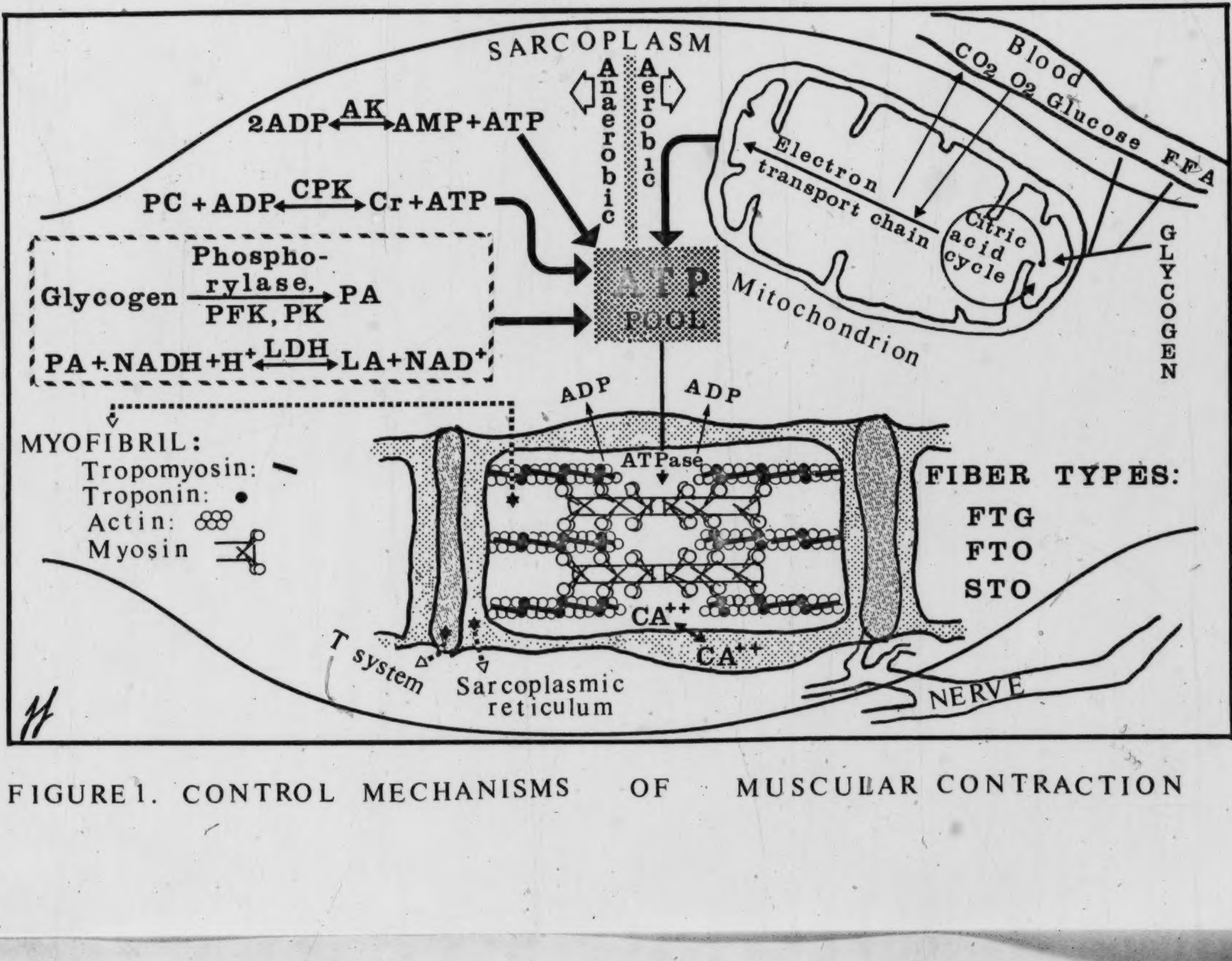

FIGURE 1. CONTROL MECHANISMS OF MUSCULAR CONTRACTION

There are two distinct forms of myosin with different myofibrillar ATPase activities (Samaha et al., 1970; Sreter et al., 1966) and contractile properties (Barany, 1967; Barnard et al., 1970 b and 1971; Close, 1972). Some authors (Barnard et al., 1970 a and 1971; Close, 1972; Edstrom and Nystrom, 1969; Engel, 1974; Muller, 1974; Peter, 1970) have used these properties to classify skeletal muscle fibers into two main groups: slow twitch (ST) or type I fibers with low ATPase activity and fast twitch (FT) or type II fibers with high myofibrillar ATPase activity. The recruitment of these fibers might be specific to the nature of the exercise training (Edington and Edgerton, 1976).

Muscular contraction is finally dependent on the availability of ATP. As ATP stores in the muscle are very limited, the resynthesis of ATP is obviously another important factor related to muscle performance. A small amount of ATP can be regenerated immediately from either phosphorylcreatine and ADP in the presence of creatine-phosphokinase (CPK, E.C. 2.7.3.2) or from ADP in the presence of myokinase or adenylate kinase (AK, E.C. 2.7.4.3). As for ATP, the stores of PC and ADP are limited and, for more prolonged work, ATP resynthesis must occur via other metabolic pathways: anaerobic glycolysis and oxidative metabolism. In the final step, anaerobic glycolysis is characterised by the reduction of pyruvate into lactate with NADH being oxidized to NAD$^+$. Although ATP resynthesis is very fast via this pathway, lactate accumulates and this has been associated with early local muscular fatigue (Ferris, 1969; Hermansen and Osnes, 1972; Keul, 1973; Margaria, 1972; Osnes and Hermansen, 1972; Wenger and Reed, 1976). Nevertheless, in sports such as wrestling, hockey and long sprint races, anaerobic glycolysis is the main energy production pathway (Astrand and Rodahl, 1970; Keul *et al*, 1972).

The pyruvate to lactate reaction is catalysed and may be regulated by lactate dehydrogenase (LDH, E.C. 1.1.1.27). LDH is composed of two subunits: a muscle specific (M) and a heart specific (H) type, which are combined in a tetramer molecule giving five isozymes (H_4, H_3M, H_2M_2, HM_3, M_4) with different properties (Cahn *et al*., 1962; Dawson *et al*., 1964; Dietz and Lubrano, 1967; Everse and Kaplan, 1970 and 1973; Kaplan, 1960-1962; Plageman *et al*., 1960a and b; Stambaught and Post, 1966a and b). M - LDH favours the reduction of pyruvate whereas H - LDH favours the oxidation of lactate.

Finally, the ATP resynthesis can occur via the oxidation of fat or carbohydrate as acetyl CoA via the citrate cycle and the electron transport chain. The aerobic energy production is the common energy source for most cellular activities in mammalian tissue, including those affected by physical exercises of low to moderate intensities.

Justification for the Study

Most physical activities can be grossly classified as one of the two following types:

1. High intensity, short duration or anaerobic*
2. Low to moderate intensity, long duration or aerobic

Sport performance is usually based on either one or both of these types of exercise. Therefore the importance of understanding the possible mechanisms of adaptations in either type of performance is obvious.

Adaptations to aerobic exercise are well documented. Maximal oxygen uptake (Astrand and Rodahl, 1970; Karlsson et al., 1967; Saltin et al., 1968), maximal cardiac output (Ekblom et al., 1968; Saltin et al., 1968), and the activities of certain oxidative enzymes (Baldwin et al., 1972; Barnard and Peter, 1971; Benzi et al., 1975; Fitts et al., 1975; Gollnick et al., 1969, 1970; Holloszy, 1967 and 1971) are all increased with prolonged exercise training executed at an intensity of greater than 50% of the maximal oxygen uptake. Cardiovascular adjustments (Bevegard and Shepherd, 1967; Dempsey and Rankin, 1967; Rowell, 1969, 1974) and substrate mobilization and/or interaction (Ahlborg, 1967; Felig and Wahren, 1975; Fredholm, 1970; Horstman et al., 1971; Issekutz et al., 1966; Jorfeldt, 1971; Jorfeldt and Wahren, 1970; Pruett, 1970; Wahren, 1970; Weil et al., 1965) in acute exercise are also well described in the literature.

---

* Anaerobic exercises are those based on ATP synthesis that do not use O_2 as the final electron carrier. In other words, the anaerobic metabolism includes the adenylate kinase, the creatine phosphokinase and the lactate dehydrogenase reactions as well as the ATP already stored and ready to be used by the muscle.

On the other hand, adaptation to anaerobic exercise and/or adaptation of the anaerobic parameters to exercise is less well documented. With the exception of blood lactate response (Issekutz et al., 1965, 1966; Jorfeldt, 1971; Margaria et al., 1933, 1968, 1972), and plasma enzyme response to exercise (Bloor, 1969; Doty et al., 1971; Fowler et al., 1962, 1968; Garbus et al., 1964; Haralambie, 1972; Hunter et al., 1971; Papadopoulos et al., 1968; Raven et al., 1970; Rose et al., 1970 a and b), few studies on the adaptation of anaerobic parameters to exercise, particularly those using anaerobic exercise, exist.

Glycogen stores have been shown to restrict long lasting muscular effort (Hultman, 1967; Piehl, 1974; Saltin et al., 1973; Taylor et al., 1971). A specific depletion pattern in ST and FT fibers with aerobic and anaerobic exercise does occur (Gollnick et al., 1973 a ; Piehl, 1974; Saltin et al., 1973). Phosphorylase (Baldwin et al., 1973; Bylund et al., 1977; Edgerton et al., 1970; Gould and Rawlinson, 1959; Holloszy et al., 1971; Kowalski et al., 1969; Morgan et al., 1971; Saubert et al., 1973; Taylor et al., 1972; Zika et al., 1971), hexokinase (Baldwin et al., 1973, 1977; Barnard and Peter, 1969; Bylund et al., 1977; Holloszy et al., 1971; Suominen and Keikkinen, 1975), phosphosfructokinase (Baldwin et al., 1973, 1977; Gollnick et al., 1973; Henricksson and Reitman, 1976; Holloszy et al., 1971; Morgan et al., 1971; Saubert et al., 1973; Thorstensson et al., 1976), and pyruvate kinase (Baldwin et al., 1973; Bostrom et al., 1974; Holloszy et al., 1971; Morgan et al., 1971; Saubert et al., 1973) activities in muscles have been shown to increase or remain stable with different types of exercise training. Many of the previously cited authors have used these parameters as indicators of anaerobic adaptation. The above mentioned enzymes (e.g. PH, PFK, HK and PK) are solicited whether pyruvate is converted to lactate or is oxidized through the citric acid cycle and the electron transport chain. On the other hand, LDH, CPK and AK activities as well as ATP and PC stores appear to contribute to non-oxidative energy production without being involved in aerobic breakdown of fuel. LDH is often seen as a regulatory enzymes of anaerobic metabolism (Everse and Kaplan, 1973; Fritz, 1965; Karlsson et al., 1974; Sjodin, 1976).

Training may induce an increase in the high energy compounds of the heart (Gangloff et al., 1961) and skeletal muscles (Ericksson et al., 1973; Gale and Nagle, 1971; Haralambie, 1972; Rogozkin, 1976), but the literature is not conclusive (Gale and Nagle, 1971; Haralambie, 1972; Karlsson et al., 1972; Saltin and Karlsson, 1971; Thorstensson et al., 1975). There may be an optimal form of exercise that leads to increased stores of ATP and PC, but this optimal form of exercise has not yet been determined. Age might also be at the origin of some reported increases (Ericksson et al., 1973).

Very few studies have dealt with the response of CPK activity to training. Some have reported increases in tissue CPK (Kendrick-Jones and Perry, 1965; Wagner and Critz, 1970) and others have noted no changes with training (Bohmer, 1969, Gangloff et al., 1961; Oscai and Holloszy, 1971; Rawlinson and Gould, 1959; Thorstensson and Karlsson, 1974). Newsholme and Start (1973, p. 113) believe that CPK may amplify the control of glycolysis when coupled with the ATPase reaction resulting in an increase in inorganic phosphate which stimulates PFK activity.

Studies showing an increase in AK activity with chronic exercise (Collowick as quoted by Kendrick-Jones and Perry, 1965; Thorstensson and Karlsson, 1974) have been reported. On the other hand, others have observed no change with training (Oscai and Holloszy, 1971). The AK reaction appears to be involved in the control of glycolysis via PFK: the small and transient decrease in ATP must be accompanied by a relatively greater increase in AMP to stimulate PFK (Newsholme and Start, 1973, p. 113).

Skeletal muscle LDH activities have been shown to remain constant (Bohmer, 1969; Gollnick et al., 1967; Hickson et al., 1976; Holloszy, 1971; Molé et al., 1973; Peter, 1970) or to decrease with training in animals (Baldwin et al., 1972, 1973; Hickson et al., 1976; York et al., 1974). In the heart, LDH activity is usually increased with training (Gollnick et al., 1961 and 1967; Walpurger and Anger, 1970; York et al., 1975 and 1976). The literature is equivocal with regard to the response to training of the LDH subunits or isoenzymes. Some authors have reported no change (Molé et al., 1973) while others have indicated increases in the H or the M type of LDH depending upon whether the training was primarily aerobic or anaerobic in nature (Karlsson et al., 1975; Peter et al., 1970 and 1971; Sjodin, 1976).

The conflicting literature may be a reflection of the fact that most studies have been looking at the effects of endurance training (aerobic type usually) on aerobic and anaerobic parameters and that few studies have looked at the specific effects of both aerobic and anaerobic training on the anaerobic parameters. The physiological age of the subjects during the training regimens and the choice of sampled tissues may have added to the confusion. Finally, the assay techniques used in many studies, particularly those investigating the LDH and high energy compound response to training, might be at the origin of some of the discrepancies reported in the literature. In some cases, the discrepancies are unexplained, a fact which further justifies the reinvestigation of these parameters.

The Problem

The purpose of this study was to determine the comparative effects of chronic, moderately intense, continuous running and high speed interval running on the following: ATP and PC stores, AK, CPK and LDH activities and LDH subunit distribution in heart (H), liver (L) and resting skeletal muscles, namely, soleus (S), medial and lateral gastrocnemius (GM , GL), plantaris (P) and tibialis anterior (TA).

Limitations and Delimitations of the Study

1. The study was confined to Sprague Dawley rats,
2. The training regimens started at 6 weeks of age and lasted 6 months, covering both the growth and adult periods,
3. The two long-term training regimens were intended to primarily overload the aerobic and anaerobic systems separately,
4. The physical activity used in the training regimen was limited to running on a motor-driven treadmill,
5. Sampled tissues were limited to the heart as an index of cardiovascular involvement, the liver as the locus of many biochemical substrate transformations and several skeletal muscles of the lower limbs to provide a picture of the adaptations of

different muscles involved in the work (agonist vs antagonist, slow twitch vs fast twitch),

6. Biochemical assays were conducted in vitro on whole muscle homogenates rather than using muscle fiber type as an index. However, the fiber composition of the chosen muscles was identified (Table 20 in Discussion),
7. Although the training programs used in this study have been shown to induce in situ improvement of the fatigue curve (Barnard and Peter, 1971) and of the oxidative capacity of the muscles (Barnard and Peter, 1971; Fitts et al., 1975; Gollnick et al., 1970), this was not verified in the present study. The only common measures used to check the effectiveness of the training regimens were body and organ weights,
8. Except for LDH, no glycolytic, glycogenolytic, or oxidative substrate or enzyme activities were measured.

CHAPTER II

REVIEW OF LITERATURE

Lactate Dehydrogenase

LDH (E.C. 1.1.1.27) catalyses the following reaction:

$$\text{pyruvate} + \text{NADH} + \text{H}^+ \underset{}{\overset{\text{LDH}}{\rightleftharpoons}} \text{lactate} + \text{NAD}^+$$

This reaction is the last and only specific step of anaerobic glycolysis. LDH is composed of two subunits: a muscle specific (M) and a heart specific (H) type. M and H subunits are combined in a tetramer molecule giving five isoenzymes:* H_4, H_3M, H_2M_2, HM_3 and M_4 which are also respectively identified as LDH-1 to LDH-5. When isolated with electrophoresis, LDH-1 is the fastest moving band toward the anode (Dietz and Lubrano, 1967; Everse and Kaplan, 1973; Plageman et al., 1960a and b). Figure 2 from Rosalki (1969) illustrates the classical separation of LDH isoenzymes in serum and tissues. Isoenzyme H_4, found in highly aerobic tissue, has a relatively low turnover number with pyruvate and is maximally active only at low concentrations of this substrate, which strongly inhibits the enzyme at higher concentrations. On the other hand, in relatively more anaerobic tissues, isoenzyme M_4 favors the pyruvate to lactate reduction for

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* "Isozyme" is also used in the literature, but the term "isoenzyme" is recommended by the Standing Committee on Enzyme of the International Union of Biochemistry (Wilkinson, 1970).

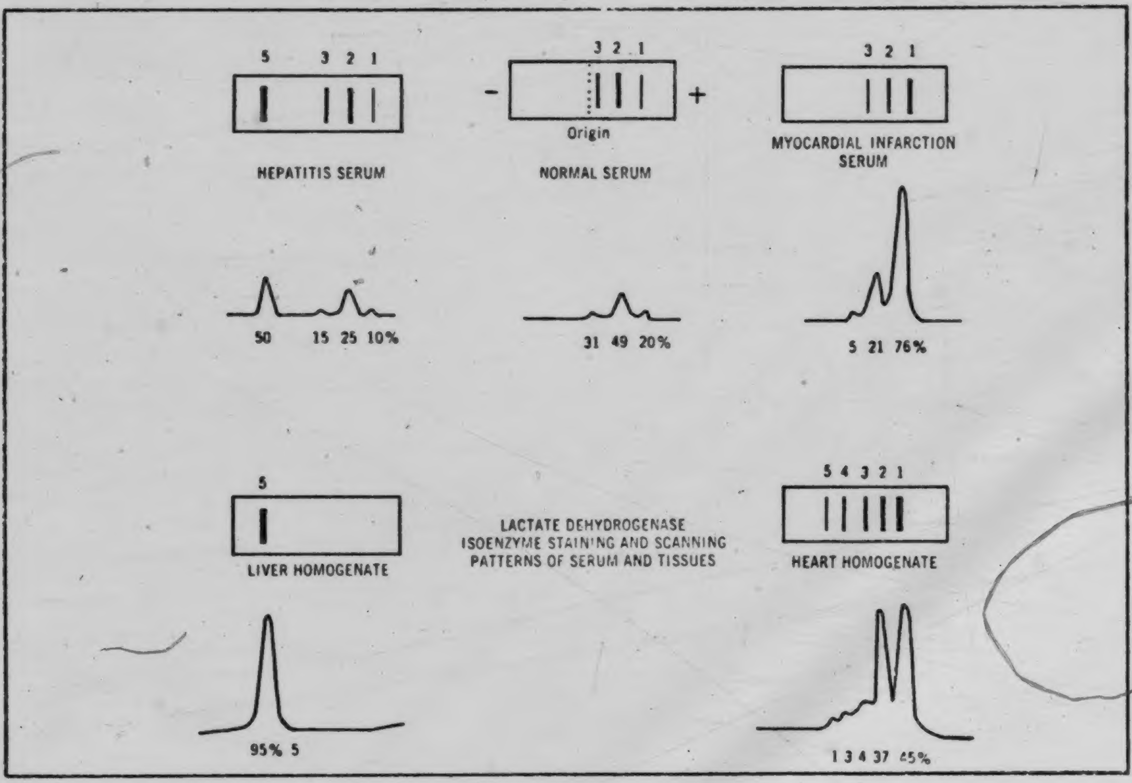

FIGURE 2. GENERAL PATTERN OF LDH ISOENZYMES (FROM ROSALKI, 1969)

immediate release of energy. Between H_4 and M_4, hybrid isoenzymes are found with proportional intermediate characteristics (Everse and Kaplan, 1973; Kaplan et al., 1961; Karlsson et al., 1974; Rosalki, 1969; Sjodin, 1976).

The M and H composition of LDH appears to be controlled by two separate and independent genes. The synthesis of M subunits is stimulated during hypoxic conditions and suppressed with high O_2 tension, while the reverse is true for the H subunits (Cahn et al., 1962; Dawson et al., 1964; Everse and Kaplan, 1973; Karsten et al., 1973; Latner and Skillen, 1968, p. 85; Thorling and Jensen, 1966). Embryologic development of M and H LDH differentiation does exist toward their future functional role. Foetal rabbit gastrocnemius appears to develop the heart muscle enzyme first, whereas the skeletal type develops postnatally (Dawson et al., 1964). Brain LDH isoenzyme patterns show more M-LDH for humans living at high altitude (Hellung et al., 1973). Muscles which contract tonically* or rythmically have relatively more H-LDH, while muscles which contract phasically have more M-LDH (Dawson et al., 1964). Function seems to be more important than tissue type, e.g. migrating birds have more H-LDH in their breast muscle than domestic birds (Wilson et al., 1963).

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* Posture and anti-gravimetric muscles (e.g. soleus, flying muscles) and the heart contract tonically and rythmically, whereas other muscles that are used only occasionally are said to be phasic muscles. This functional classification corresponds to the fast twitch (phasic) and slow twitch (tonic) muscles.

Other kinds of adaptations have been reported. For instance, hormonal or vitamin treatment (Acebal et al., 1974; Beitner et al., 1973; Dawson et al., 1964; Garbus et al., 1964; Hirota et al., 1976), sideropenic anemia (Penney et al., 1974) and denervation (Dawson et al., 1964) have been shown to induce LDH isoenzyme changes. It is interesting to note that 10 days after sciatic section, only a slight decrease in enzyme activity had occured and it is only after 31 days that M-LDH decreased in rabbit leg muscles. Such adaptations may be tied to the disappearance of the anaerobic stimuli. Duration is obviously an important factor to control when studying isoenzyme adaptation. Intermittent long-term stimulation (8 hr/day) of fast rabbit tibialis anterior up to 28 days with a frequency pattern resembling that of a slow muscle (10 impulses/sec) resulted in a decrease of total LDH activity with concomitant decrease in M-LDH % (Pette et al., 1973).

The Aerobic-Anaerobic Theory. According to Kaplan's group (Cahn et al., 1962; Dawson et al., 1964; Everse and Kaplan, 1973; Everse et al., 1970) and to Pfleiderer's group (Pfleiderer and Wachsmuch, 1961, as quoted by Latner and Spillen, 1968, p. 80 and Thorling and Jensen, 1966), LDH of the H type (heart) has evolved to operate as a regulator of pyruvate metabolism in highly aerobic cells and functions as a lactate dehydrogenase because it is inhibited by high pyruvate concentration; LDH of the M type (skeletal muscle and liver) has evolved to prevent this inhibition in anaerobic tissue and operates as a pyruvate reductase. Wuntch, Vesell and Chen (1970a), Vesell and Pool (1966) and Amarasingham and Uong (1968), have contested this theory and suggested other functions than metabolic ones for LDH isoenzymes. First, they pointed out that liver with its high M-LDH content is a highly aerobic tissue which is, however, in opposition to Pfleiderer's findings (Latner and Spillen, 1968, p. 80). Second, they were unable, at high LDH concentrations similar to cellular concentrations ($7x10^{-6}M$) to show the classical inhibition of H-LDH to high pyruvate concentration obtained with the usual hundred fold dilution of homogenate procedure. They suggested other metabolic roles for LDH isoenzymes: 1) a regulatory function for LDH-5 M_4 considered as an allosteric protein, 2) an association of isoenzymes in different proportions with various subcellular particles, in particular LDH-5 within the nucleus, 3) predominance of LDH-5 in rapidly dividing cell or in tissue capable of rapid cell proliferation, and 4) a conservative metabolic role in which one isoenzyme would be required to maintain critical enzymatic function in a tissue where another isoenzyme was rapidly degraded.

Latner _et al_., (1966) were unable to reproduce the above experiment. Similarly, Everse, Berger and Kaplan (1970, 1973) and Stambaugh and Post (1966) obtained LDH-1 usual inhibition at high enzyme concentrations with high pyruvate concentrations. According to them, inhibition is due to the formation of an abortive ternary complex between pyruvate, nicotinamide-adenine dinucleotide (NAD^+) and the LDH. They obtained different results because the pyruvate substrate was added to the LDH-NADH solution in the presence of NAD^+ whereas the other groups (Amarasingham and Uong, 1968; Vesell and Pool, 1966; Wuntch _et_ _al_., 1970a) excluded the NAD^+ resulting in the LDH reaction without inhibition because the fast reaction occured before any ternary complex was formed.

Wuntch, Chen and Vesell (1970b) further stated that the inhibition in vivo is also diminished with competition by other enzymes (glyceraldehyde -3- phosphate dehydrogenase, malate dehydrogenase) or albumin with NAD^+. These authors (Vesell and Pool, 1966 and Wuntch _et_ _al_., 1970b) noted that pyruvate in vivo never reaches levels high enough to inhibit LDH and that NAD^+ is probably more determinant of the LDH reaction.

However, Kaplan and collaborators (Everse and Kaplan, 1973) still believed that the binding of NAD^+ to other proteins in vivo, resulting in a net decrease in the concentration of the free NAD^+, does not affect the concentration of free NAD^+ in the cell to such an extent that formation of the abortive LDH complex is no longer feasible. They also added that the results obtained by Vesell and his co-workers could be due to the limiting amount of NAD^+ that was used in their experiments (14.0 μM). Another possible explanation for these discrepancies may be that a significant part of H-LDH is present in an inactive form in the fresh tissue extracts (Everse and Kaplan, 1973).

Stambaugh and Post (1966a) reported that H-LDH inhibition in the forward reaction is more a matter of product (i.e. lactate) inhibition than substrate (i.e. pyruvate) inhibition with in vivo concentrations. Karlsson (1971a) has shown that muscle pyruvate concentration increased from 0.06 at rest to 0.13 mmoles/kg wet weight at maximal work load; even maximal pyruvate concentration found in vivo is about 10 times lower than that required to produce the substrate inhibition reported by Kaplan's group (Everse and Kaplan, 1973; Kaplan _et_ _al_., 1960, 1962, 1968). On the other hand, Everse _et_ _al_., (1970) did show substrate inhibition at physiological enzyme concentrations. The possibility that the intracellular concentration of the pyruvate at the actual locations of the isoenzymes may reach inhibitory levels could not be excluded but was not considered likely (Vesell and Pool, 1966; Wuntch _et_ _al_., 1970b). Although it seems that there exists no study on LDH inhibition with in vivo concentrations for all the reagents, most authors believe in some kind of inhibition in vivo (Everse _et_ _al_., 1970; Everse and Kaplan, 1973; Karlsson _et_ _al_., 1974b; Latner and Skillen, 1968; Sjodin, 1976; Stambaugh and Post, 1966).

To understand the functional role of LDH isoenzymes, one must realize that pyruvate, lactate, NADH and NAD$^+$ compete together to bind with LDH and either form the ternary abortive complex or the ternary complex that catalyses the reaction. It seems that pyruvate/lactate and NAD$^+$/NADH ratios are more important than the absolute concentrations of these substrates and products.

Table 2, reproduced from Everse and Kaplan (1973), illustrates the probable role of H-LDH with different NAD$^+$/NADH and PA/LA ratios. H-LDH appears to be under metabolic control and is regulated by its own oxidized products as well as the oxidized coenzymes. Therefore, in normal concentration, H-LDH is prevented from reducing pyruvate to lactate. Lactate can be produced by the heart during ischemia and myocardial infarction due to the lack of oxygen supply and the NADH increase which displaces the abortive complex with H-LDH and favors the reduction of pyruvate. Reactions 2 and 3 (Table 1) appear to be geared to assure an optimal concentration of reduced coenzyme under various physiological conditions.

The aerobic-anaerobic theory is in accordance with isoenzyme distribution in muscle fibers and cellular localizations. M-LDH is more predominant in fast twitch fibers and H-LDH, in slow twitch fibers (Blanchaer and Van Wijhe, 1962; Brody and Engel, 1964; Fine et al., 1963a; Karlsson et al., 1974b; McMillan, 1967; Peter et al., 1971; Sjodin,

TABLE 2 Effects of Substrate and Coenzyme Concentrations on H-LDH in Aerobic Tissues.*

Reaction No.	Concentration Ratios NAD$^+$/NADH	Pyruvate/Lactate	Action of H-LDH
1	high	high	Formation of E-NAD$^+$-pyruvate abortive complex**
2	high	low	Oxidation of lactate (E-NAD$^+$-lactate)
3	low	high	Reduction of pyruvate (dissociation of E-NAD$^+$-pyruvate complex as in myocardial infarction (E-NADH-pyruvate)
4	low	low	Formation of E-NADH-lactate complex

* From Everse and Kaplan, 1973.

** E = Enzyme (H-LDH)

1976a; Van Wijhe et al., 1964). The higher LDH activity in fast twitch fibers, as demonstrated biochemically or histochemically (Karlsson et al., 1974b; McMillan, 1967; Meijer, 1973; Peter et al., 1971; Sjodin, 1976a), seems to be related to higher M-LDH content, although this was not the case for endurance trained athletes (Karlsson et al., 1975) due to the absence of a linear relation between total LDH and ST fibers (Karlsson et al., 1975). It is interesting to note that most LDH is located in the sarcoplasm (Brody and Engel, 1964; Sjodin, 1976a), but LDH isoenzymes with predominant H subunits are also located in mitochondria, and LDH isoenzymes mainly composed of M subunits have a strong binding affinity for membranes, e.g. sarcoplasmic reticulum or external mitochondrial membrane (Brody and Engel, 1964; Sjodin, 1976) or other subcellular particulate fractions (Ratner et al., 1974). Specific LDH isoenzyme compartmentalization within subcellular units may increase the difficulty with which NADH reaches LDH and may be related to or explain some physiological function attributed to LDH isoenzymes (Ratner et al., 1974; Sjodin, 1976a).

LDH as a Regulatory Enzyme of Glycolysis. As shown previously, LDH may play an important role in controlling, the amount of lactate produced and in oxidizing NADH accumulated in the cell. But it seems that this is not under the exclusive control of LDH. According to Boxer and Devlin (1961) and Keul et al. (1972, pp. 14, 87, 131), two other shuttle reactions can assure the oxidation of NADH. First, dihydroxyacetone-P is reduced to alpha-glycerophosphate in the sarcoplasm with the glycerophosphate dehydrogenase; the cycle is closed in the mitochondria by the reverse reaction. Second, acetoacetate is reduced to beta-hydroxybutyrate in a similar way. Like lactate, alpha-glycerophosphate increases in anaerobic states, but it is not a "dead end" product like lactate and its significance in terms of fatigue is unknown.

Actual evidence does not suggest, however, that endurance training preferentially accentuates the glycerophosphate dehydrogenase compared to the LDH since changes were of the same order of magnitude and in the same direction for both enzymes (Baldwin _et_ _al_., 1973; Morgan _et_ _al_., 1971). Baldwin _et_ _al_. (1973) reported a decrease from 4 (n.s.) to 27% ($P < 0.02$) and 15 ($P < 0.05$) to 23.3% ($P < 0.001$) for the alpha-glycerophosphate dehydrogenase and the LDH respectively in the red and white quadriceps of the rat: in the soleus alpha-glycerophosphate dehydrogenase and LDH increased respectively by 54.7% ($P < 0.001$) and 12% (n.s.). Morgan _et_ _al_. (1971) reported non significant decreases of 26.9% and 22% for alpha-glycerophosphate dehydrogenase and LDH of human quadriceps after endurance training. Holloszy and Oscai (1969) had previously found similar results. In rat heart, Kraus (1971) reported a 85% increase either with a strenuous swimming program and a voluntary running program. Staudte _et_ _al_. (1973) reported no change in either LDH or alpha-glycerophosphate dehydrogenase after sprint training in the rat. According to Boxer and Devlin (1961), the NADH shuttles can work only in aerobiosis or partial anaerobiosis since they are based on citric acid cycle intermediates. As the direct oxidation of cytoplasm formed NADH in the mitochondria is not possible, only pyruvate can oxidize NADH in anaerobiosis.

Concerning the regulation of LDH itself, it seems that the mass action law is not sufficient to explain muscle lactate formation since pyruvate and lactate do not increase at the same rate in exercise (Karlsson, 1971a; Keul et al., 1967 and 1972). According to Fritz (1965), M_4-LDH but not H_4-LDH behaves like an allosteric enzyme and is also activated by the seven citric acid cycle intermediates as well as by aspartic and glutamic acids which are directly converted to citric acid cycle intermediates. Although there was a significant increase (50%) in malic enzyme (L-malate: NADP oxidoreductase, E.C. 1.1.1.40) after endurance training in the rat (Molé et al., 1973), the very low absolute levels of this enzyme do not seem to play an important role in the pyruvate metabolism (e.g. lipogenesis) in skeletal muscle (Molé et al., 1973).

According to Felig and Wahren (1971, 1973 and 1975), another pathway, pyruvate conversion to alanine, may interact with the LDH reaction, decreasing the pyruvate* available for oxidation of NADH via anaerobic glycolysis. According to these authors, the alanine pathway is a non-toxic alternative to ammonia in the transport of amino groups from the periphery to the liver, where alanine is converted back to glucose. The lower muscle or blood lactate levels observed in submaximal exercise in trained individuals (Astrand and Rodahl, 1970, p. 379; Karlsson, 1968, 1971a; Molé et al., 1973; Robinson et al., 1941) may be explained by an increase in glutamate pyruvate transaminase (GPT, E.C. 2.6.1.2.) and more pyruvate being converted to alanine (Felig and Wahren, 1971, 1975; Molé et al., 1973).

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* There are other alternative fates for pyruvate (Malher and Cordes, 1966, p. 435), but their importance and functional role in exercise is presently unknown.

However, at maximal and supra-maximal work loads (e.g. $\dot{V}O_2$max or maximal voluntary contraction), trained subjects have higher lactate accumulation (Astrand and Rodahl, 1970, p. 379; Ericksson et al., 1973; Molé et al., 1973; Robinson et al., 1941), suggesting a greater contribution of LDH and probably of M-LDH. Reciprocal behaviour in lactate formation at low and medium work load compared to high work load appears to be ruled by the inhibition of lactate and free fatty acids on each other at these work loads (Issekutz et al., 1965, 1966; Felig and Wahren, 1975; Fredholm, 1970; Horstman et al., 1971; Molé et al., 1973; Weil et al., 1965).

Lactate can be used as substrate by the heart (Everse et al., 1973; Keul, 1971, 1973), the liver (Keul, 1973; Rowell, 1966, 1971) and more or less by the skeletal muscles (Issekutz et al., 1965; Jorfeldt, 1971; Keul, 1971; Felig and Wahren, 1975). Lactate diffuses from the organs where it is produced (mainly the skeletal muscle) to the blood (Jorfeldt, 1971; Keul, 1967, 1971, 1973a and b; Margaria, 1968, 1972). When there is a sudden rise in anaerobic metabolism, a delay is observed before equilibrium is reached between the blood and the muscle. The "peak" blood lactate is usually reached between 3 to 10 minutes after exercise (Karlsson, 1971a; Margaria, 1968, 1972). Blood and muscle lactate concentrations are also dependant on the equilibrium between uptake and production. The peak lactate in the blood, although smaller than the muscle concentration, is always representative of the muscle concentration (Karlsson, 1971a). It is worthwhile to note that blood lactate reflects an average situation for all the muscles of the body taken together, even though lactate may be found in different amounts in different muscles or even in different muscle fibers (Essen and Haggmark, 1975) where possible product inhibition can occur (Karlsson et al., 1971, 1975; Sjodin, 1976a and b).

High lactate concentrations have been associated with exhaustion in exercise of high intensity (Ahlborg, 1972; Karlsson, 1971a; Keul, 1973, Margaria, 1972, 1968). Lactate may be either a cause or consequence of fatigue, but it has been suggested (Ferris, 1969) that the lactate anion itself is related to anxiety and other similar symptoms. Others, (Hermansen and Osnes, 1972; Keul, 1973; Osnes and Hermansen, 1972) suggested that the increase in acidity linked with lactate production may be the cause of fatigue. There seems to be a good correlation between lactate concentration and exhaustion feelings, at least in short lasting-high intensity work. According to Margaria (1968, 1972) and Keul (1973), anaerobic glycolysis has the second fastest energy production rate after the immediate utilization of high energy phosphate stores. Therefore, when less intense work is done, energy can be produced via oxidative pathways and exercise can be performed longer before exhaustion is reached. With prolonged exercise, however, lactate concentration and production is low and cannot be related to exhaustion (Karlsson, 1971a; Keul, 1973).

Lactate is produced and accumulates when the energy demand is greater than the energy that can be produced with aerobic metabolism. In supra-maximal exercise, lactate is produced to a greater extent than it is taken up and this is reflected by the greater accumulation of lactate in the muscle and the blood. At low work loads (30% $\dot{V}O_2$max), lactate is probably produced but does not accumulate (Di Prampero et al., 1976; Margaria, 1968, 1972). At medium intensity (30-60% $\dot{V}O_2$max), which can be sustained for a long time, lactate increases at the beginning but returns to resting levels with time (Jorfeldt, 1971; Felig and Wahren, 1973; Karlsson et al., 1968; Keul et al., 1972). Even maximal work after prolonged exercise does not produce the usual lactate rise (Astrand, 1963).

To explain the sudden rise of lactate at the beginning of medium work load, Jorfeldt (1971) and Felig and Wahren (1975) suggested a net release of lactate from white fibers which are mainly composed of M-LDH. When the delay due to circulatory adjustments is finished, the red fibers are able to meet the energy demand aerobically and assume the responsibility of muscle contraction, perhaps using lactate as a substrate. It is known that during prolonged exercise, glycogen depletion as measured at 20, 60, 120 and 180 mm of exercise, first occurs in ST fibers but as the exercise progresses, the FT fibers are also depleted (Gollnick et al., 1973d). But this fiber specific depletion pattern could not be observed before 60 min of exercise and is not necessarily in contrast with the early transient lactate rise that was observed at the 20 min mark (Gollnick et al., 1973d). During repeated 1 min sprints of high intensity interspaced with 10 min of rest, glycogen is first depleted in FT fibers as opposed to the glycogen depletion pattern of prolonged exercise (Gollnick et al., 1973a). Others (Baldwin et al., 1973; Piehl, 1974) have reported the possibility of a selective recruitment pattern of different fiber types at the onset of work and with various types of exercise. Essen and Haggmark (1975) recently measured lactate in single muscle fibers* and in pooled muscle fibers of the same type in exercises known to result in lactate formation. With single fiber measurements, they found great variations in post exercise lactate, both for type I and type II fibers reflecting a selective fiber pattern. Lactate from pooled or single fibers was higher in some cases for type II fibers.** The exercise stimuli used in this study were bicycle work at 100% VO_2max and static contraction at 50% of MVC which, if the selective recruitment theory is good, should preferentially involve fast twitch or type II fibers. It would be interesting to have a similar study with exercise of medium intensity where lactate is known to increase at first and to decrease thereafter.

Isoenzyme Pattern Modifications. The crucial and central point of glycolytic regulation by LDH is the LDH subunit or isoenzyme pattern and its possible modification with physiological demand (e.g. training). According to Millar (1974), lactate dehydrogenase must be in an "activated state" to hybridize in vivo. Simple dissociation to dimers is not enough for hybridization. In vivo, therefore, an environment must be created in which the enzyme is not membrane bound, in which the anti-hybridizing effects of substrate, coenzyme and protective ions are prevented from occurring and in which "activation" can take place. These are highly restrictive conditions. The possibility exists that, in vivo, activating agents are present which negate the influence of the inhibitors and accelerate hybridization.

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* Single muscle fibers were dissected from freeze-dried samples and the ends cut off for identification with myosin ATPase reaction. Single fibers were placed directly in a fluorometric tube for analysis. Pooled fibers (25-50 fibers of the same type) were first digested in HCL and the supernatant analysed conventionally.

** No statistical analyses were made in this preliminary study.

Newly and preferentially synthesized LDH subunits appear to be the route by which isoenzyme patterns are modified. The half-life of LDH may be as long as 31 days in rat skeletal muscle and somewhat shorter in the liver and the heart (Fritz et al., 1969). Opposite trends have been shown for the time of occurrence of peak specific radioactivities (Don and Master, 1975; Fritz et al., 1969). Heart M_4 has been shown to possess a much shorter half-life than any other isoenzyme in the heart, the liver or the skeletal muscle of the rat (Fritz et al., 1973).

Acute Exercise and LDH

In serum, LDH and more specifically M-LDH may temporarily increase by as much as 400% in trained and untrained humans and animals if the relative intensity and duration of the work load are sufficient (Block et al., 1969; Bloor and Papadopoulos, 1969; Doty et al., 1971; Fowler et al., 1962; Garbus et al., 1964; Hallonen and Konttinen, 1962;

Haralambie, 1972; Hunter and Critz, 1971; Novosadova, 1969; Papadopoulos _et_ _al_., 1968; Raven _et_ _al_., 1970; Rose _et_ _al_., 1970a and b; Schmidt and Schmidt, 1969; Siest and Galteau, 1974; Wolfson et al., 1972). Many mechanisms, including cellular necrosis, membrane disruption, increased permeability due to hypoxia or to increased circulating catecholamines, increased blood flow, carriers, etc... have been proposed to explain the increased release of LDH and M-LDH from tissue to serum (Atland and Highman, 1961; Doty _et_ _al_., 1971; Garbus _et_ _al_., 1964; Highman and Altland, 1963; Karlsson _et_ _al_., 1968; Raven _et_ _al_., 1970; Sanders and Bloor, 1975; Schmidt and Schmidt, 1969; Siest and Galteau, 1975; York _et_ _al_., 1976). Such a release from tissue must rely on some assumptions. For instance, tissue LDH either maintains the same activity but its concentration is decreasing as a result of cellular leakage to serum or increases its activity or its concentration with a concomitant increase in serum. Cellular leakage is not directly related to LDH activity itself and thus does not seem to be related to a possible training effect in tissue LDH. Such a decrease in rat tissue LDH after acute exercise has been reported by Doty _et_ _al_. (1971). Novosadova (1969) reported that heart, liver and skeletal muscle LDH decrease in trained and untrained rats with acute exercise but serum LDH, paradoxically, increased in untrained rats and decreased in trained rats. Other trends have however been reported for tissue LDH after acute exercise. Garbus _et_ _al_. (1964) observed no consistent changes. Gollnick et al. (1967) reported no significant change in LDH activity of rat heart or skeletal muscle after acute exercise. Finally, Karlsson et al. (1968) reported a significant increase in human muscle LDH after prolonged exercise. The reasons for these different findings are unclear at the present time. Karlsson et al. (1968) believed that the increase in muscle LDH was due to an increased enzyme concentration and possibly to a change in the Michaelis constant. Due to the long half-lives of LDH (Fritz et al., 1969, 1973), it seems that such changes must result from a transient change in activity (inhibition or facilitation). This reported increase in human muscle LDH after strenuous prolonged exercise appears to be paradoxical and is unique in the literature. It seems paradoxical because 1) such prolonged exercise is known to yield low lactate levels (Astrand et al., 1963; Jorfeldt, 1971; Felig and Wahren, 1973; Karlsson et al., 1968; Keul et al., 1972), 2) LDH activity is well above the maximal rate of lactate formation in vivo (Karlsson et al., 1968), 3) training with prolonged exercise does not increase human skeletal muscle LDH (Bylund et al., 1977; Morgan et al., 1971; Sjodin et al., 1976a and b), 4) and all other human and animal studies showed similar increases in serum LDH after acute exercise and all other animal studies* either showed a decrease or no change in tissue LDH after acute exercise. Species differences are not excluded. In this regard, it is interesting to note that in rat serum about 80% of the LDH is present as M-LDH (Bloor and Papadopoulos, 1969; Garbus et al, 1964; Raven et al., 1970) as compared to 20-30% for human serum (Barengo and Itoiz, 1972; Block et al., 1969; Dietz and Lubrano, 1967; Rose et al., 1970). Acute effects of exercise on LDH appear to be transitory and quite independant of chronic effects of exercise. Thus the acute effects of exercise on LDH are not a very useful aid to understanding the chronic effects of exercise.

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* A recent preliminary study (Sjodin, 1976a) indicated that LDH and M-LDH did not change in human muscle after a 100 km race.

Chronic exercise and LDH

Although the relationship between serum and tissue LDH is puzzling, training does effectively reduce the LDH rise in serum at a particular submaximal work load (Bloor and Papadopoulos, 1969; Garbus et al., 1964; Hunter et al., 1971; Novosadova, 1969; Papadopoulos et al., 1968; Rose et al., 1970a; Wolfson et al., 1972) and at rest (Hallonen and Konttinen, 1962). Another study has reported, however, a mild but significant increase in resting serum after training (Hunter and Critz, 1971).

In tissues, LDH changes appear to be related to the type of training as well as to the type of tissue. Endurance training, either running or swimming, appears to increase the LDH activity of the heart (per mg of N_2 or mg of fresh tissue) by 10 to 30% (Gollnick et al., 1961, 1967; Walpurger and Anger, 1970; York et al., 1975 and 1976) and more specifically, the M-LDH* activity by 22 to 30% as well as the M-LDH% by 3 to 5% (Peter, 1970; York et al., 1975 and 1976). The LDH increase in the heart may be a function of the intensity and duration of training as well as of the age at which the training regimen was started (York et al., 1975 and 1976). Two other studies (Peter, 1970; Ruhling et al., 1973) reported nonsignificant changes in total LDH activity of the heart after endurance training.

In skeletal muscles, LDH adaptation to endurance exercise appears more complex and more confusing. Generally, fast twitch skeletal muscle of endurance trained athletes have 30 to 68% less LDH activity than sedentary subjects (Costill et al., 1976; Karlsson et al., 1975; Suominen and Heikkinen, 1975). Genetic endowment may explain these differences since none of the endurance training studies in humans showed significant changes in mixed skeletal muscle (Bylund et al., 1977; Morgan et al., 1971; Sjodin et al., 1976a and b). In each case studied, however, there was a decrease of 6 to 22%. Most training studies in animals also showed decreases of 7 to 58% in fast twitch skeletal muscles. These trends were often non significant in the mixed FG and FOG gastrocnemius and plantaris (Böhmer, 1969;

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* In many cases, M-LDH activity and M-LDH% have been estimated from isoenzyme activities or % using the following formula:

$$\text{M-LDH} = 0.25\ H_3M + 0.5\ H_2M_2 + 0.75\ HM_3 + M_4$$

Gollnick *et al*., 1967; Hickson *et al*., 1976; Holloszy, 1971; Molé *et al*., 1973) and sometimes significant in FG muscles (Baldwin *et al*., 1972, 1973; Hickson *et al*., 1976; York *et al*., 1974). Two studies reported no change (Gollnick and Hearn, 1961; Gould and Rawlinson, 1959) and another reported a 39% nonsignificant increase in rat biceps brachii with tonic training (Zika *et al*., 1973). In slow twitch muscle, like the soleus, there may be an opposite trend with an increase of about 12% in LDH activity after endurance training on a treadmill (Baldwin *et al*., 1972b and 1973). Hickson *et al*. (1976) have, however, reported a small but significant decrease in soleus LDH using a running wheel device. It was also found that FOG muscles had a greater decrease in LDH activity than FG muscles (Baldwin *et al*., 1973; York *et al*., 1974).

The effects of sprint training on tissue LDH are less well documented and appear even more confusing than the effects of endurance training. Fast twitch skeletal muscles of sprint and strength trained athletes have significantly higher LDH and M-LDH activity than endurance trained athletes and sedentary persons (Costill *et al*., 1976; Karlsson *et al*., 1975). Although present, none of the increasing trends were significant in sprint training studies dealing with humans (Sjodin *et al*., 1976b; Thorstenson *et al*., 1975). Hickson *et al*. (1976) did report a significant 15-20% decrease with sprint training in fast twitch skeletal muscles of rats whereas Staudte *et al*. (1973) reported no change after a 21 day sprint training study. In the heart, Ruhling et al. (1973) found no myocardial LDH change with sprint training. Isometric training that brought exhaustion in 4 to 5 min, 3 times twice a day for 25 days in a row with at least 30 min recovery between each exercise bout, had no effect on LDH activity of fast twitch rectus femoris of female rats but decreased the LDH activity in the soleus (Exner et al., 1973a). In male rats, there were no change in LDH activity of either rectus femoris or soleus after a similar isometric training (Exner et al., 1973b).

To summarize, endurance training appears to decrease LDH and M-LDH activity in fast twitch skeletal muscle, and to increase these activities in the heart and soleus. The changes in LDH are a function of the fiber type composition of the muscles. On the other hand, the few sprint training studies reported either indicate a similar or an opposite LDH behaviour in mixed skeletal muscle of humans and other mammals.

The decrease in LDH of fast twitch skeletal muscle with endurance training could be explained by the increased oxidative capacity of these muscles (Baldwin et al., 1972) and by the greater contribution of aerobic metabolism to energy demand. It has been shown that during endurance training more pyruvate is converted to alanine or is directed toward the citric acid cycle and more fat is oxidized (Felig and Wahren, 1975; Gollnick et al., 1969, 1970, 1972;

Holloszy, 1971; Molé _et_ _al_., 1973). Therefore, less energy is coming from the LDH reaction, particularly from the M-LDH reaction, and the observed LDH decrease might be a secondary side effect of the increasedoxidative capacity. On the other hand, the increase of LDH in the heart, which has been attributed to the most anaerobic subunit (i.e. M-LDH) is surprising, particularly in view of the fact that endurance training increases the utilisation of lactate as a substrate (Keul, 1973a). This heart specific adaptation is not exclusive to LDH, and is consistent with other findings. In this regard, it is interesting to note that Hearn and Gollnick (1961) reported increased ATPase activity in the heart but not in the gastrocnemius of endurance swim-trained rats. Heart muscle also usually shows less oxidative adaptation to endurance training than skeletal muscle (Baldwin _et_ _al_., 1977a; Holloszy, 1975; Oscai _et_ _al_., 1971b).

It is known that LDH half-lives were estimated to 1.6, 16 and 31 days in the heart, liver and skeletal muscle respectively (Fritz et al., 1969). In addition, M_4 isoenzyme of the heart has a much shorter half-life than any other isoenzyme of the heart, liver or skeletal muscle (Fritz _et_ _al_., 1973). These facts are consistent with the heart LDH and M-LDH activity changes, but could not explain the long term adaptation that resulted from a 6 month training regimen.

York _et_ _al_. (1975 and 1976) have shown that experimental hypoxia resulted in M-LDH concentrations twice as large as those found after running or swimming, in both ventricles and atria, even though hypertrophy was present only in the right ventricle and atria. Hypoxia appears more important for M-LDH synthesis than hypertrophy and its accompanied cellular and chemical changes. If hypertrophy per se would increase the activities of LDH, as has been hypothesized (York et al., 1975), one would expect relative increases in other glycolytic enzyme activities. To date, this phenomenon has not been demonstrated in the literature (Baldwin et al., 1973; Gollnick and Hermansen, 1973; Saubert et al., 1973; York et al., 1975). Hypertrophy might still explain partially, at least, some of the LDH increases in the heart. In this regard, Walpurger and Anger (1970) reported significant heart LDH increases and hypertrophy only in the running training group and not in the swimming group.*

Therefore, hypoxia appears more important than hypertrophy to explain LDH and M-LDH increases in the heart. Such hypoxic changes would be consistent with the aerobic-anaerobic theory previously described and other reports on the effects of hypoxia on LDH (Dawson et al., 1964; Fox and Reed, 1969; Hellung et al., 1973; Thorling and Jensen, 1966). Since oxygen delivery to the heart might not be limited during moderate sustained exercise (Holloszy, 1973; Rowell, 1974; York et al., 1975 and 1976), York et al. (1975 and

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* Baldwin et al. (1972a, 1977a and b) reported that swimming usually resulted in greater heart hypertrophy than running in rats.

1976) believed that M-LDH could increase in the absence of hypoxia but gave no alternative explanation for this behavior. The situation is somewhat similar to the oxidative enzyme rise after aerobic training since O_2 tension in muscle appears more than adequate (Rowell, 1974; Stainsby, 1973) and where the oxygen uptake, even at maximal rate, is well below the oxidative enzyme activities (Holloszy, 1967 and 1971).* Thus, the oxidative enzymes as well as LDH appear to adapt to a situation where the energy demand is increased or the oxygen availability is decreased even though it does not reach a critical level. In skeletal muscle, the oxidative enzymes appear to adapt first to the demand resulting in a decrease in LDH. In the heart, the oxidative enzymes adapt to a lesser extent (Baldwin _et al._, 1977a; Holloszy, 1975; Oscai _et al._, 1971b) maybe because oxidative enzyme levels in the heart are already in a trained state and closer to a possible physiological limit so that the extra energy demand of training has to be met by increased LDH activity. The increase in the soleus LDH activity is harder to explain on the hypoxia basis. It is possible that this slow twitch muscle might have been under a greater stess than fast twitch muscles if equally solicitated by the running action. It is known that glycogen is depleted faster in ST fibers as compared to FT fibers in prolonged exercise (Gollnick et al., 1973a , d).

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* The oxygen supply as a limiting factor to muscular contraction is still an open question however (Bostrom _et al._, 1974; Kaijser, 1970 and 1973).

Those facts might indicate a possible rôle of anaerobic glycolysis in this muscle.

Hypoxia might be a stimulus for LDH adaptation but it is hard to reconcile the facts that on one side trained heart oxidizes more lactate in moderate exercise and on the other side, has higher LDH and M-LDH activity. The fact that other glycolytic enzymes of the heart do not increase and that more fat is being oxidized might be at the origin of the LDH changes. Trained skeletal muscles also use more lactate as substrate and training might reduce the circulating lactate.

With sprint training which does not increase the VO_2max (Sjodin et al., 1976b and Thorstenson et al., 1975), an increase in LDH activity seems to be mandatory to meet the energy demand. The fact that animal studies did not support such an adaptative pattern may be due to the insufficiency of the training programs. Hickson et al. (1976) used only 10 sec work intervals and 40 sec rest intervals. Staudte et al. (1973) used longer work intervals (45 sec) but their rats did only 4 repetitions a day and trained only for 21 days.

Other reasons seem to warrant further investigation on the effect of chronic exercise on LDH activity and LDH subunits. As endurance and sprint training might have opposite effects on LDH, one form of these exercises has to be used exclusively. The use of mixed regimens (e.g. sprint intervals superimposed to endurance running) might explain some of the nonsignificant results reported earlier (Holloszy, 1971; Molé et al., 1973) and should be avoided. Since many of these studies were significant only when studying the quadriceps muscles (Baldwin et al., 1972a and 1973; Hickson et al., 1976; York et al., 1974) and not significant when dealing with the gastrocnemius or the plantaris (Böhmer, 1969; Gollnick et al., 1961, 1967; Hickson et al., 1976; Holloszy, 1971 ; Molé et al., 1973), there may be specific muscle recruitement that should be further investigated. Gould and Rawlinson (1959) have reported no LDH change in the rat biceps brachii after swimming training, but it is not clear that this muscle is a prime mover for this motion. A biochemical comparison of "agonist" and "antagonist" muscles appears necessary. Finally, in many of the reviewed studies, total LDH activity was often measured at only one pyruvate concentration and M-LDH% was estimated without the previous determination of the species-specific optimal pyruvate concentrations for M-LDH and H-LDH. This may explain some of the many nonsignificant but large percentage changes reported in the literature.

Metabolism of High Energy Compounds*

ATP is the immediate source of energy for muscular contraction (Cain et al., 1962; Davies, 1971; Maréchal, 1972; Mommaerts, 1969). ATPase catalyses the conversion of ATP into ADP. Because ATP stores are very limited (Davies, 1971; Karlsson, 1971a), ATP has to be resythesized continually to pursue muscular contraction. Most newly resynthesized ATP comes from oxidative metabolism and anaerobic glycolysis. In addition, minute amounts come from the conversion of PC and ADP into ATP through the two following reactions:

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* See footnote on next page.

$$\mathrm{PC} + \mathrm{ADP} \xrightleftharpoons{\mathrm{CPK}} \mathrm{Creatine} + \mathrm{ATP}$$

and

$$2\ \mathrm{ADP} \xrightleftharpoons{\mathrm{AK}} \mathrm{AMP} + \mathrm{ATP}$$

Adenosine Triphosphate and Phosphorylcreatine

Energy liberated from the reverse of PC is the most rapidly available (Cain _et_ _al_., 1962; Di Prampero _et_ _al_., 1970; Hohorst _et_ _al_., 1962; Hultman _et_ _al_., 1967; Karlsson, 1971a; Keul _et_ _al_., 1972; Margaria, 1972; Piiper and Spiller, 1970). PC breakdown seems to provide most of the energy necessary for a 6 to 20 seconds work bout of high intensity like a 100 meter run (Keul, 1973; Margaria, 1968, 1972).

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* Ennor and Morrisson (1958) have reviewed the origin of the term "Phosphagen" and suggested that "Phosphagens" should be regarded as a generic name embracing all (and restricted to) those naturally occurring phosphorylated guanidine compounds which function as stores of phosphate-bond energy from which phosphoryl groups may be transferred to ADP to form ATP as a result of enzymatic catalysis. Thus ATP itself is not a phosphagen as it is often implied (Cerretelli and Di Prampero, 1969; Gollnick and Hermansen, 1973; Karlsson, 1971a; Pernow and Saltin, 1971). In addition, phosphorylcreatine is preferred to phosphocreatine or to creatine phosphate since it does contain a phosphoryl group (creatine-PO_3H_2) and not a phosphate group ($-PO_4H_2$). Thus, "High Energy Compounds" or "Energy Rich Compounds" expressions appear more adequate to describe the combination of ATP and PC compounds than the term "Phosphagens".

Even with limited stores, ATP and PC initial levels have a definite effect on this kind of sport performance. PC stores are depleted very soon at work (Cain et al., 1962; Davies, 1971; Di Prampero et al., 1970; Ericksson et al., 1973; Hohorst et al., 1962; Hultman et al., 1967; Karlsson et al., 1971a; Keul et al., 1972; chapt. III; Knutgen and Saltin, 1973; McGilvery and Murray, 1974) whatever the working intensity (Di Prampero et al., 1970; Karlsson, 1971a; Keul et al., 1972, chapt. III). ATP levels are usually maintained at equilibrium or more or less depleted in very severe work of if CPK is inhibited (Cain et al., 1962; Davies, 1971; Hohorst et al., 1962; Hultman et al., 1967). Most PC stores are replenished very rapidly, within 2 minutes in man or animals (Fox et al., 1969; Hultman et al., 1967; Margaria 1968, 1972; Piiper and Spiller, 1970).

Other facts support the importance of high energy compounds on contractile performance. Borredon (1967) showed a negative correlation between PC levels of the heart and electrocardiographic ST depression. Feinstein (1962), Fox and Reed (1966) and Rabinowitz and Zak (1975) reported up to 24 and 54% decrease in ATP and PC with experimental congestive heart failure. Total tension developed in the anaerobic state seems to be related to ATP and PC levels (Cerretelli and Di Prampero, 1969; Harris et al., 1975). Running performance in rats was reduced when the creatine reserve was itself decreased with a synthetic creatine analog diet (Shields et al., 1975). Cairella and Vecchi (1966) and

Cier (1965) reported an increased swimming endurance time in rats after ingestion of PC supplements.

Many experimental conditions - PC,K^+ and Mg^{++} Aspartate, amino-Acids, Monosodium Phosphate, vitamin C administration - have been shown to increase ATP and PC levels (Pourel, 1968). Rabinowitz and Zak (1975) recently reviewed several forms of cardiac hypertrophy (e.g. acute cardiac overload, developing cardiac hypertrophy, compensated cardiac hypertrophy) and found a decrease or no significant changes in ATP and PC. Degenring et al. (1975) and Scheuer et al. (1970) reported similar results for endurance swimming training in rat heart. In 1961, Gangloff et al. reported a paradoxical 32% increase in rat heart after endurance treadmill training, but the values (e.g. 1.5 mmole/kg wet weight) were well under the usual reported range (5-11 mmole/kg wet weight). Many of the results reported before 1960 are low due to poor sampling and extraction techniques (Wollenberger et al., 1960) and thus, are questionable.

In the skeletal muscles, the effect of physical training on ATP and PC stores is somewhat confusing. According to Yakolev (1965) and Yampolskaya (1952) (as quoted by Haralambie, 1972), PC levels increased up to 75% after training with short exercises of high intensity as compared to lower intensity training. Russian workers are currently assessing the efficiency of their training programs with the specific PC response to training (Rogozkin, 1976). There may be an opposite trend in PC adaptation in heart and skeletal muscle (Harren, 1938, quoted by Haralambie, 1972). Other studies, however, are no more conclusive. Compared to sedentary rats, Gale and Nagle (1971) found that neither sprint nor endurance running-trained rats had significantly different ATP and PC levels of the soleus and gastrocnemius plantaris muscle group. Absolute values reported by these authors were only about 50% of the usual values. Saltin and Karlsson (1971) studied the effect of physical conditioning in man on ATP and PC but reported no significant change on these parameters. Karlsson _et_ _al_. (1972) showed that endurance training increases ATP but not PC levels of the quadriceps. Thorstensson _et_ _al_. (1975) did not show any significant change in ATP and PC after short sprint training in man. Swimming also failed to alter significantly the ATP levels of rat gastrocnemius (Böhmer, 1969).

Ericksson _et_ _al_. (1973) reported an 11 and 39% increase of ATP and PC respectively in vastus lateralis of 11-13 years old boys after 6 months of general conditioning. They explained the ATP increase by the concomitant mitochondrial increase but could not explain the unusual increase in PC. It is interesting to note that the pre-training values were 14.5 as compared to 20.2 mmole x kg^{-1} wet weight after training. Scandinavian groups (Bergstrom _et_ _al_., 1971; Harris _et_ _al_., 1974; Hultman _et_ _al_., 1967; Karlsson _et_ _al_., 1971a) usually have reported values of 17 to 21 mmole x kg^{-1} wet weight in human adult quadriceps. Since there was no control group for age effect, it may be that the increase reported by Ericksson et al. (1973) reflected an aging effect rather than a training effect. Casten (1950) and Quarto di Palo (1960) previously reported an increase in PC but not in ATP in maturing rats.

To summarize, it seems that training induces a decrease in ATP and PC levels of the heart. In the skeletal muscle, the situation is not conclusive and requires further investigation. Some authors (Ericksson et al., 1973; Jacobs and Klingenberg, 1964; Haralambie, 1972; Keul et al., 1972, chapt. III; McGilvery and Murray, 1974; Rabinowitz and Zak, 1975; Saks et al., 1974; Seraydarian et al., 1974) questioned the physiological importance of possible ATP and PC changes. They believe that enzyme activities (CPK and AK) are more efficient ways to adapt to the energy demand and that PC may be more important as a regulator of other energy synthetic reactions or as a form of energy that can be transported from the mitochondria to the myofibrils rather than being a storage form of energy.

Creatine phosphokinase

Creatine phosphokinase (CPK) is a dimer composed of a brain type (B) and a muscle type subunit (M), but the nature and the function of the three isoenzymes (BB, BM, MM) is very unclear (Traugott et al., 1973). As the amount of brain type CPK is usually very low in muscle (Saks et al., 1974), more concern is given to total CPK.

The effects of acute exercise on serum CPK are well documented (Block et al., 1969; Fowler et al., 1962 and 1968; Haralambie, 1972, 1973; Hunter and Critz, 1970; Kendrick-Jones and Perry, 1965; Nuttall and Jones, 1968; Rose et al., 1970; Sanders and Bloor, 1975; Schmidt and Schmidt, 1969; Siest and Galteau, 1971; Wagner and Critz, 1970). It seems that serum CPK may increase up to 400% (Wagner and Critz, 1970) and is a better index of the work load intensity than other enzymes (Sanders and Bloor, 1975). Kendrick-Jones and Perry (1965) and Wagner and Critz (1970) reported a muscle CPK rise after acute exercise or in vitro contraction (isometric or isotonic) but this could not be confirmed by Oscai and Holloszy (1971) and Dieter (1970) unless there were Vit C deficiencies. On the other hand, Bostrom et al. (1974a and b) reported a decrease in tissue CPK after swimming or in vitro stimulation (isometric or isotonic). Serum and tissue changes after acute exercise are generally transient and have probably little significance for the understanding of the chronic effects of exercise on tissue CPK.

The effects of chronic exercise on tissue CPK appear inconsistent from one report to another. Endurance training has been shown to increase the animal heart, soleus and gastrocnemius CPK by more than 10% (Wagner and Critz, 1970). Other studies (Böhmer, 1969; Dart and Holloszy, 1969; Dieter, 1970; Oscai and Holloszy, 1971; Rawlinson and Gould, 1959; Walpurger and Anger, 1970) reported no CPK change in either mitochondrial or cytoplasmic extracts of heart or skeletal muscles. Bohmer (1969) and Kendrick-Jones and Perry (1965) showed a CPK decrease in human and animal muscles after immobilisation, but this does not imply a tissue CPK increase after training. As a matter of fact, Suominen and Heikkinen (1975) and Thorstensson *et al*. (1974, 1976a and b) failed to show a CPK increase in human muscle after endurance and strength training respectively. On the other hand, Thorstensson *et al*. (1975) found a 35% increase in human muscle after sprint training. In animals, sprint training resulted in a 12% CPK increase in the soleus but did not change the CPK activity of the rectus femoris (Staudte *et al*., 1973), whereas isometric training had a reciprocal effect in these two muscles, both in male and female rats (Exner *et al*., 1973).

To summarize, tissue CPK does not seem to change with training except perhaps with sprint and isometric training. Further research is required to substantiate CPK adaptation to chronic exercise.

Adenylate Kinase

ATP can be resynthesized through the adenylate kinase (AK) reaction. According to Newsholme and Start (1973), the energy provided by this reaction can suffice for 3 seconds at most. From the athlete's point of view, this could be very important. For biochemists, however, this is a very small amount of energy as compared to other energy sources. Newsholme and Start (1973) considered this reaction as an amplification mechanism for the regulation of glycolysis since the AK reaction is alway close to equilibrium and the ATP concentration is 50 times larger than the AMP concentration. Relatively small changes in ATP are amplified by the relatively large change in the AMP effector. Other functions have been attributed to AK, such as a rephosphorylation of the adenylic acid accumulating with the oxidation of the fatty acid (Pette, 1971) or an extra intramitochondrial adenosine nucleotide exchange (Klingenberger, 1965). Thus, the biochemical function of AK is not clearly determined. Adenylate kinase adaptations to chronic exercise are not more conclusive. Oscai and Holloszy (1971) indicated no change in mitochondrial and cytoplasmic adenylate kinase after endurance running in rat gastrocnemius. In heart muscle, however, Walpurger and Anger (1970) found a 50% and a 30% rises in cytoplasmic, but not in mitochondrial, AK after endurance swimming and running in the rat respectively. Dart and Holloszy (1969) failed however to demonstrate any AK adaptation after experimental heart hypertrophy in the rat using arteriovenous fistula. In human skeletal muscles, Thorstensson _et_ _al_. (1975 and 1976b) failed to show any AK change after sprint and strength training although they observed a 7.8% significant increase with strength training in a previous study (Thorstensson _et_ _al_., 1976a).

In conclusion, it seems that skeletal muscle AK changes rarely with training, except maybe with strength training. There is also a possible increase in the heart AK activity.

CHAPTER III

METHODS AND PROCEDURES

Animals

Forty male Sprague Dawley rats (Canadian Breeding Farm and Laboratories, Ltd., St. Constant, Quebec) were used in the study. The rats were approximately six weeks of age and weighed 160 ± 17 grams (X ± SD) at the time of arrival. The animals were placed in 60 X 60 X 30 cm self-cleaning cages at 25°C, ≈50% relative humidity and 755 mm Hg in groups of ten for the first 8 weeks and in groups of five thereafter. Cage locations in the rack were rotated in a random fashion once a week. Rats were exposed to the usual 12hr daylight and 12hr darkness. Rats were identified with a color code on the proximal end of the tail with the use of a marking pen. Rats were fed ad libitum with tap water and standard rat chow (Charles River Rat and Mouse Formula) containing 22% protein, 5% fat, 5% fiber and 11% moisture.

Experimental Groups

After an initial exercise program, the thirty best runners were numbered in order by weight and randomly assigned to one of three experimental groups*:

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* At that time, the rats were relocated according to their new group, always 10 per cage. This resulted in aggressive behaviour which was somewhat reduced by putting 5 rats per cage. Individual cages were unavailable due to restricted facilities.

Sgr: Sedentary group

Cgr: Continuous training group

Igr: Intermittent training group

Mean group weights of the rats (X ± SD) were 305.0 ± 26.5, 317 ± 18.0, 311.7 ± 20.4 grams for Sgr, Cgr and Igr respectively at time of group assignment. Due to the limited availability of the "trainer", running time had to be changed a few times during this 6 month experimental period. On a few occasions, training was conducted in the evening with the lights on. At all other times, the rats were trained in day light either in the morning at 8:00 or at the end of the afternoon at 17:00. The attrition rate for the experimental animals was 2 of 10 per group.

Initial Exercise Program. After one week of only cage activity for adaptation to the new environment, an initial exercise program, consisting of running five days per week for four weeks, was provided. Running took place on a motor-driven treadmill accomodating 10 animals at a time and incorporating a shock grid at the rear of the compartments to motivate the animals to keep pace with the belt movement (Quinton Instruments, Seattle, Wash. U.S.A., Small Animal Treadmill, Model 42-15). The rats learned to run continuously for 6 minutes on an 8% grade with the speed progressively increased from 10 to 31 m/min. This progression is low enough to avoid significant training effects on the activities of the oxydative enzymes (Benzi _et_ _al_., 1975; Fitts _et_ _al_., 1975; Holloszy, 1967; Molé _et_ _al_., 1973).

In addition, following the continuous work, the initial exercise program included low intensity intermittent running, starting with 10 X 15 sec work at 15m/min with 15 sec rest intervals and finishing with 10 X 15 sec work at 35-40 m/min with 10 sec rest intervals. The initial training program was deleted thereafter for the entire 6 month experimental period. Knowing the reversible effect of training, it was assumed that the initial training program would have no training effect at the time of sacrifice.

The sedentary group (Sgr) was restricted to normal cage activity: eating, drinking, sleeping, walking, fighting and weekly weighing.

The continuous training group (Cgr) ran for 6 months, five days per week. The treadmill was set at 31 m/min and 8% grade. The duration of the training session was gradually increased from 10 to 50 minutes over 3 months, maintained at this level for 1.5 month and reduced to 40 minutes for the last 1.5 month.* This training stimulus is believed to be the maximal that can be handled by these rats. It was therefore impossible to reproduce Holloszy's training regimen (1967) as originally intended. Holloszy was able to have young Wistar rats running for 2 hours after 3 months and demonstrated significant changes in the oxidative capacity of the rats (Baldwin _et al_., 1972; Holloszy, 1967; Molé _et al_., 1973; Pattengale and Holloszy, 1967). Nevertheless, Fitts _et al_. (1975) and Gollnick _et al_. (1970) have reported intensive glycogenolytic and lipolytic responses in rats with a training regimen similar to the one used in this study.

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* The training load had to be reduced because the rats failed to run after 40 minutes even with increased electrical stimulation. This behavior may be linked to the cage re-assignment that followed the initial exercise program since the rats were harder to train at that time. Reduced training had also been reported by others (Barnard and Peter, 1971; Peter, 1970).

The intermittent training program (Igr) was designed to cause a greater effect on the anaerobic metabolism. Consequently, the intensity was higher than for the continuous training. Each training session started with a 5 minute warm-up period at 31 m/min and 8% grade. The rats ran 10 X 1 minute at an intensity that was gradually increased from 40 to 75 m/min over the first 5 months and maintained at 70 m/min (8% slope) during the 6th month. Each work interval was interspaced by a 4 minute rest interval.

Dependent Variables

The dependent variables mostly represent key metabolites or enzymes of the anaerobic metabolism in different tissues. These include:

1. Absolute weight of the body or the organs, W_{abs}. These measures provide a simple check of the effectiveness of the training programs;

2. Relative weight of the organ, W_{rel}. The ratio of organs W_{abs} over body W_{abs} is used as a parameter per se as well as a relative index of organ weight since the usual decrease of body weight with training may compensate for the expected organ hypertrophy. For the second purpose, regressed weights (Gollnick et al., 1967; Héroux and Gridgeman, 1958; Muller, 1975; Tanner, 1949) are usually better than W_{rel}, but will not be reported here since no significant correlations between organ weights and body weights were observed in this study. In addition, it has been reported that W_{rel} is similar to the regressed weight of muscles (Héroux and Gridgeman, 1958; Muller, 1975);
3. Adenosine triphosphate, ATP;
4. Phosphorylcreatine, PC;
5. ATP + PC;
6. Creatine phosphokinase, CPK (E.C. No. 2.7.3.2, ATP: creatine phosphoryltransferase);
7. Adenylate kinase, AK (E.C. No. 2.7.4.3, ATP: AMP phosphotransferase);

8-13. Lactate dehydrogenase, LDH (E.C. No. 1.1.1.27, L-Lactate: NAD oxidoreductase);

8. LDH activity at 21×10^{-4}M PA, LDH_{21}; at high pyruvic acid concentration, there is inhibition of the heart type of LDH subunit;

9. LDH activity at $3x10^{-4}$M PA, LDH_3; at low pyruvic acid concentration, there is inhibition of the muscle type of LDH subunit;
10. The ratio of LDH_{21} over LDH_3, LDH_{21}/LDH_3, is a good indicator of the percentage of heart and muscle types of LDH subunits, an important aspect of anaerobic metabolism since "H" type favors the lactate to pyruvate reaction whereas the "M" type favors the pyruvate to lactate reaction;
11. LDH activity related to muscle type of LDH subunits, M-LDH;
12. LDH activity related to heart type of LDH subunits, H-LDH;
13. Total LDH activity = M-LDH + H-LDH, LDH.

The dependent variables as well as their sampling sites are listed in Table 3.

Sampling Procedures

At the end of the training period, rats were sacrificed at rest, two days after the last work bout to avoid acute exercise effects on dependent variables.

The measurement of ATP and PC is critical for the sampling procedures due to the rapid hydrolysis in anaerobic conditions (Karlsson, 1971; Lamprecht, 1963; Lowry et al., 1964a and b). Rats were anesthetized with an intraperitoneal injection of 50 mg of Nembutal (sodium pentobarbital Abbott, 50 mg/ml) per kg of body weight. This anesthetic

TABLE 3 Listing of Dependent Variables

MEASURES*	1	2	3	4	5	6	7	8
	BODY	TIBIALIS ANTERIOR	PLANTARIS	GASTROCNEMIUS		SOLEUS	HEART	LIVER
				MEDIALIS	LATERALIS			
	(BW)	(TA)	(P)	(GM)	(GL)	(S)	(H)	(L)
1. W_{abs}	x	x	x	x	x	x	x	x
2. W_{rel}		x	x	x	x	x	x	x
3. ATP		x	x	x	x	x	x	
4. PC		x	x	x	x	x	x	
5. ATP + PC		x	x	x	x	x	x	
6. CPK		x	x	x	x	x	x	x
7. AK		x	x	x	x	x	x	x
8. LDH_{21}**		x	x	x	x	x	x	x
9. LDH_{3}**		x	x	x	x	x	x	x
10. LDH_{21}/LDH_{3}		x	x	x	x	x	x	x
11. M - LDH		x	x	x	x	x	x	x
12. H - LDH		x	x	x	x	x	x	x
13. TOTAL LDH		x	x	x	x	x	x	x

* See Table 1 for abbreviations.

** LDH at $21x10^{-4}M$ and $3x10^{-4}M$ PA respectively.

was preferred to ether in order to minimize excitation and also possible changes in enzyme activities that occur with ether (Ben et al., 1969; Katona, 1973). However, Nembutal has a depressive effect on the cardiovascular system (Sawyer et al., 1971) and definite effects on substrate concentrations of rat liver (Faupel et al., 1972). ATP levels are not affected by Nembutal (Faupel et al., 1972). The effects of Nembutal on heart and skeletal muscle substrates and on enzymes are not known but the number and the sites of sampled tissues required anaesthesia of the rats. A systematic error, if any, should not disturb comparisons of the three training groups. After discarding the skin and the superficial muscle layers, the muscles of the left leg were isolated in the following order GM, TA, S, P, GL, with minimum trauma, leaving intact the circulation, innervation and insertions.

Each muscle was isolated with a small plastic plate approximately 15 cm long, 1 mm thick, and 1.5 cm wide at one end and 3 cm at the other. The plastic plate had protuberant rounded edges increasing the thickness of the plate to 4 mm. This plate assured better thermal insulation and made it easier to grasp the suspended muscle with copper tongs precooled in liquid nitrogen (-190°C). The muscle was then cut immediately along the edge of the copper tongs and put into the liquid nitrogen. Protruding tissue was broken off the copper tongs to avoid contamination with slowly frozen tissue. The samples were immediately wrapped in aluminum foil and temporarily stored in liquid nitrogen and then, in a deep freeze at -60°C (Revco, Ultralow, Model ULT-075-0-2) until chemical analysis was carried out. At these temperatures samples are stable almost indefinitely. (Lowry and Passonneau, 1972, pp. 120-122).

The tongs used were a pair of Vise-Grip pliers (Plate # 1) modified by soldering a pair of copper blocks to the original lips of the pliers in such a way that block surfaces always compressed the muscle into a parallel sheet of 1 mm thickness to ensure even, rapid and constant freezing within and between samples. Copper was preferred to the often used aluminum because it offers a slightly higher thermal conductivity and freezes 1.4 X more tissue for the same block size due to a higher density that largely compensates for its lower specific heat*. Each copper block was

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* Physical characteristics (Handbook of Chemistry and Physics, 1973).

1. Thermal conductivity (Watt/cm)

	0°C	25°C	-173°C
Aluminum	2.36	2.37	3.0
Copper	4.01	3.98	4.83

2. Specific heat (cal/g.C$^{\circ}$)
   Al: 0.215; Cu: 0.093

3. Density (g/cm^3)
   Al: 2.7; Cu: 8.9

4. $\Delta Q = mc\ \Delta t$ or $m_1 c_1 \Delta t_1 = m_2 c_2 \Delta t_2$
   where ΔQ = heat transfer (calories)
   m = mass (grams)
   c = specific heat (cal/g.C$^{\circ}$)
   Δt = change in temperature (C$^{\circ}$)

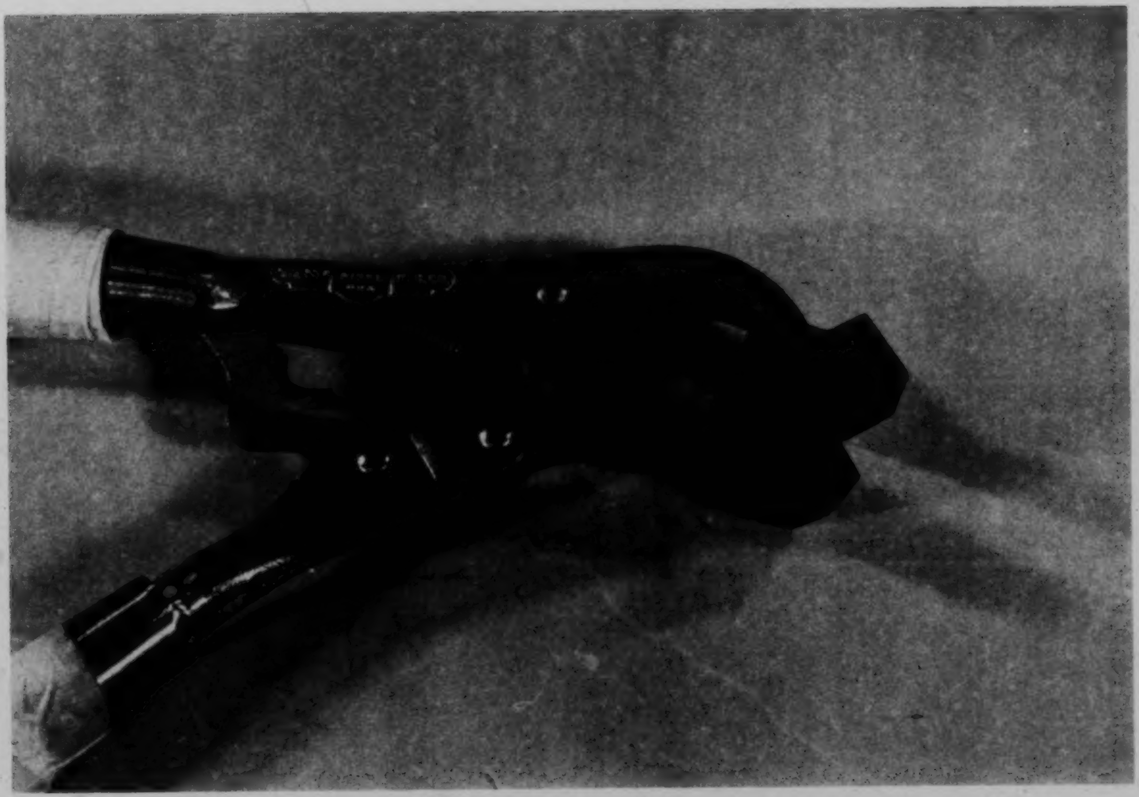

PLATE 1. MODIFIED VISE-GRIP PLIERS WITH COPPER BLOCKS USED TO FREEZE THE TISSUES IN SITU

1 cm thick; other dimensions can be deduced from its cross section (Figure 3). Such a design allowed samples of various sizes and shapes to be taken.

It is believed that such a freezing technique is the simplest and the best available (Adam, 1963a; Cartier, 1967a; Eranko, 1954, Faupel et al., 1972; Hess, 1963; Lampretch et al., 1963a and b; Leunissen and Piatnek-Leunissen, 1968; Lowry and Passonneau, 1972; Pourel, 1968; Swynghedauw et al., 1967). Compared to immersion techniques using various refrigerants, "quick-freeze" tongs yielded better results. Even for isopentane, the best refrigerant, freezing (36° to 0°C) required over 7 sec with tissue samples as small as 200 mg due to the heat isolation of immersed tissues. This is caused by the development of a gaseous wrap of refrigerant (phenomenon of Leidenfrost) and is also due to the low thermal conductivity of the tissue itself. This delay is sufficient to cause hydrolysis of ATP and metabolic changes in many other substrates (Faupel et al., 1972).

The heart was extirpated next. It has been shown that the anoxia delay due to the opening of the thorax is enough to cause hydrolysis of ATP and PC (Adam, 1963a; Lampretch et al., 1963a and b). Therefore, a tracheotomy was performed for assisted ventilation using an artificial respiratory pump, (Rodent pump, Model 680-1, Harvard Apparatus Co., Dover, Mass. U.S.A.). The thorax was then opened and the heart was lifted by its apex and flattened

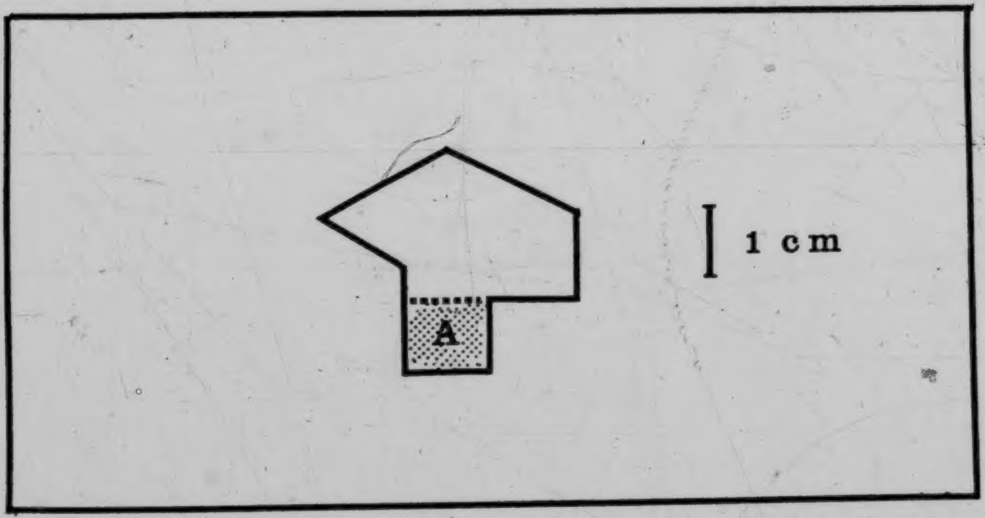

FIGURE 3. OVERHEAD DIMENSIONS OF COPPER BLOCKS USED IN FREEZING TISSUES. BLOCKS WERE SOLDERED TO THE ORIGINAL JAWS OF THE VISE-GRIP PLIERS WITH PART "A". BLOCKS WERE 1 CM THICK.

between the precooled copper tongs. The whole heart was maintained for weighing and enzyme analyses. Non-beating hearts due to poor ventilation were eliminated from ATP and PC analyses. Heart tissue was analysed similarly to the analyses mentioned previously for the left leg samples.

The liver was removed, frozen by immersion in liquid nitrogen and stored in a freezer at -60°C for further weighing and analyses. Finally, the muscles of the right leg were isolated, dissected, frozen by immersion, and stored at -60°C for weighing and enzyme analyses.

Analytical Methods

All chemicals used in this study and their source of supply are listed in Appendix G.

Tissue Preparation

Enzyme analyses. Fifty to 100 mg of wet tissue were blotted for blood, freed of connective tissue and weighed to one tenth of a milligram. Tissues were homogenized using 10 ml of o.25 M sucrose per g of tissue with a Kontes glass pestle and tubes of size AA or A (Canadian Scientific Company, Montreal, Canada, Cat. No. 885451 and 885452 for the pestles and the tubes respectively). The pestle was rotated by a motor (Fisher Scientific Co., Montreal, Canada, Dyna-Mix Model 143) at about 1000 rpm using rubber tubing as an universal joint. The tube was moved up and down manually (about 30 times to complete muscle disintegration) and frequently put in ice to prevent over-heating, (Hess, 1963). Crude homogenates were centrifuged at 4000 g and 4^oC for 10-15 min (International Equipment Co., Centrifuge, No. PR-6000). Then, to bring the absorbance changes into the region of 0.02 to 0.05 absorbance units per minute with the assay mixtures, further dilutions were made as follows:

ENZYMES	SKELETAL MUSCLE	HEART	LIVER
AK	1/100	1/100	1/100
CPK	1/10000	1/1000	1/100
LDH	1/1000	1/1000	1/1000

and 0.05 ml of these dilutions were used to start the reaction in the assay mixture. The same sample was used for AK, CPK and LDH assays.

ATP and PC analysis. Tissue preparation (weighing, deproteinization, homogenization, centrifugation and neutralization) was conducted in a 4^oC refrigerated room. Tissue samples of one rat were transferred from the freezer to a Dewar flask containing liquid nitrogen and maintained in this flask between manipulations. Until deproteinization, care was taken to limit exposure to 4^oC air to less than 10 consecutive seconds. Tissue samples (50-400 mg) were rapidly weighed on a Roller Smith balance (Biolar Corporation, Model LG, North Grafton, Mass.) to one tenth of a mg and transferred back to the Dewar flask. The deproteinization was carried out according to Lampretch _et al_. (1963a and b), using 6.5 ml of $HClO_4$ 6% W/V for 2 g of muscle tissue. Perchloric acid was preferred to trichloracacetic acid because the latter is reported to inhibit the G6P-DH used in the assay (Cartier et al., 1967a; Lowry and Passonneau, 1972, p. 123).

The exact amount of $HClO_4$ required was calculated and drawn into a pipet (Pipetman, Model P by Gibson, Analytical Instruments, Mississauga) for later use. Next the tissue was transferred to a porcelain mortar (30 ml capacity) containing liquid nitrogen and ground with a precooled porcelain pestle to a fine powder. As needed, 10 ml portions of liquid nitrogen were added to avoid complete evaporation. Then, the $HClO_4$ was slowly added and ground with the powdered tissue in liquid nitrogen. After grinding and evaporation of remaining nitrogen, the powdered mixture of muscle and $HClO_4$ were transferred to a Kontes glass homogenizing tube with a small plastic spatula and homogenized when the mixture started to melt, using the procedure described previously. Samples were left standing for 5-10 minutes to ensure complete deproteinization (Cartier et al., 1967a; Bucher et al., 1963), then centrifuged at 4°C for 10 minutes at 4000 g. Care was taken to minimize the time of deproteinization. The tolerance time for 0.6 M $HClO_4$ at 0°C is 1 hour for acid labile ATP and PC (Hess, 1963; Lowry and Passonneau, 1972, p. 124).

Supernatants of centrifuged homogenates were transferred to small test tubes, and neutralized to pH 7.4 according to Lamprecht (1963a and b) using Methyl Orange as the indicator and K_2CO_3 (5M) as titratant. Samples stood for 10 min. to permit $KClO_4$ sedimentation. The supernatant was transferred to another tube to avoid contact with any acid stable enzymes such as MK and ATPase in the sediment that might have affected PC or ATP assays. From this final solution, 0.05 or 0.1 ml was added quickly for the assay, because there is a slight hydrolysis of ATP and CP on standing (Lamprecht, 1963a).

Assays

All enzyme assays were conducted at 30° C, using 1 cm square cuvets containing 3 ml of reagent solution and recording the % transmittance change for 2 minutes at a wave length of 340 nm on a Beckman spectrophotometer (Model DB-6) coupled to a Fisher recorder (Recordall, Model 5223-51). Transmittance readings were transformed to absorbance and to enzyme activities using a small desk computer (Hewlett Packard, No. 9801 A).

Adenylate kinase was measured according to Oliver's method (1955). The reactions are basically:

$$\text{ADP} \xrightleftharpoons{\text{AK}} \text{ATP} + \text{AMP} \tag{1}$$

$$\text{ATP} + \text{glucose} \xrightleftharpoons{\text{HK}} \text{ADP} + \text{G6P} \tag{2}$$

$$\text{G6P} + \text{NADP} \xrightleftharpoons{\text{G6P-DH}} \text{6-phosphogluconate} + \text{NADPH} + \text{H}^+ \tag{3}$$

According to Newsholme and Start (1973), the forward reaction is the fastest and is preferred to the reverse one (Collowick, 1955; Kleine and Chlond, 1967).

Creatine phosphokinase was measured according to Oliver's method (1955) as modified by Nielsen and Ludvigsen (1963) and Rosalki (1967), using commercial kits (Dade, CPK-UV-1 or CPK-UV-10) which have proven to be reliable and valid (Crowley and Alton, 1970; Rosalki, 1967). The reactions of this method are:

$$\mathrm{PC} + \mathrm{ADP} \xrightleftharpoons{\mathrm{CPK}} \text{creatine} + \mathrm{ATP} \qquad (1)$$

$$\mathrm{ATP} + \text{glucose} \xrightleftharpoons{\mathrm{HK}} \mathrm{ADP} + \mathrm{G6P} \qquad (2)$$

$$\mathrm{G6P} + \mathrm{NADP} \xrightleftharpoons{\mathrm{G6P\text{-}DH}} \text{6-phosphogluconate} + \mathrm{NADPH} + \mathrm{H}^+ \qquad (3)$$

According to Rosalki (1967), the forward reaction is faster and is preferred to the reverse reaction.

Lactate dehydrogenase assay was more complicated. By measuring activity at two predetermined pyruvate concentrations, it is possible to obtain the percentage of "H" and "M" monomers of the LDH, plus activity due solely to "M" and "H" monomers or to total LDH. The method was essentially that used by Dawson and Kaplan (1964), Fox and Reed (1969), Hirota et al., (1976), Kaplan and Cahn (1962), Latner and Skillen (1968, p. 80), Plagemann et al. (1960a and b) and Stambaugh and Post (1966a). Thus, with two simple spectrophotometric assays, it is possible to obtain not only enzyme activity but also relative distribution of

"H" and "M" monomers. This method yields essentially the same information as the more complicated electrophoretic separation of the LDH isoenzymes since an equal mixture of LDH_5 (i.e. M_4) and LDH_1 (i.e. H_4), results in the same total activity as LDH_3 (i.e. M_2H_2) (Everse, 1973, p. 66; Kaplan and Cahn, 1962; Latner and Skillen, 1968, p. 30; Plagemann et al., 1960b).

LDH catalyses the following reaction:

$$PA + NADH + H^+ \xrightleftharpoons{LDH} LA + NAD^+ \qquad (1)$$

This LDH method is based on the specific calalytic properties of the relative composition in "H" and in "M" monomers. Each monomer has a different optimal pyruvate concentration for maximal velocity of the reaction. Therefore, at the two optimal pyruvate concentrations, the total velocities of the reactions are the following:

at optimal PA concentration for M-LDH,

$$V_1 = M\text{-}LDH + x\ H\text{-}LDH \qquad (1)$$

and at PA concentration for H-LDH,

$$V_2 = y\ M\text{-}LDH + H\text{-}LDH \qquad (2)$$

The optimal pyruvate concentrations as well as the values of x and y must be determined for each species (Cahn et al., 1962; Fine et al., 1963a; Latner and Skillen, 1968, p. 4, 20, 35). In this study, rat M and H were isolated with polyacrylamide gel electrophoresis using the method of Dietz and Lubrano (1967). Plate 2 (Appendix A) is an example of the isoenzyme separation. Additional heart and skeletal muscle samples were treated in a similar fashion except for the staining procedure. The fastest and the slowest moving bands toward the anode, H_4 and M_4 respectively, were cut according to two stained samples run at the same time.* Then these unstained discs of M_4 and H_4 LDH were spectrophotometrically analysed to determine the optimal pyruvate concentrations (Figure 6 and Table 21 in Appendix A). Hence, it was found that the optimal pyruvate concentrations were the following:

$$PA = 21 \times 10^{-4}M \text{ for } M_4 \text{ or M-LDH}$$

$$PA = 3 \times 10^{-4}M \text{ for } H_4 \text{ or H-LDH}$$

The percentage of enzyme activity at these two concentrations were as follows:

PA	M_4	H_4
$21 \times 10^{-4}M$	100%	80%
$3 \times 10^{-4}M$	77%	100%

Therefore, equation (1) and (2) were re-written as:

$$LDH_{21} = 1.00\ M + 0.80\ H \tag{3}$$

$$LDH_3 = 0.77\ M + 1.00\ H \tag{4}$$

From (3) and (4), the ratio LDH_{21}/LDH_3 (V_1/V_2) which also indicated the percentage of H and M subunit (Figure 4), the activity solely due to H and M subunits (H-LDH and M-LDH) and the total LDH (i.e. M-LDH + H-LDH) can be computed.

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* The ratio of the distance covered by the marker dye to that covered by the isoenzyme band was used. This ratio was constant for the same batch of analyses.

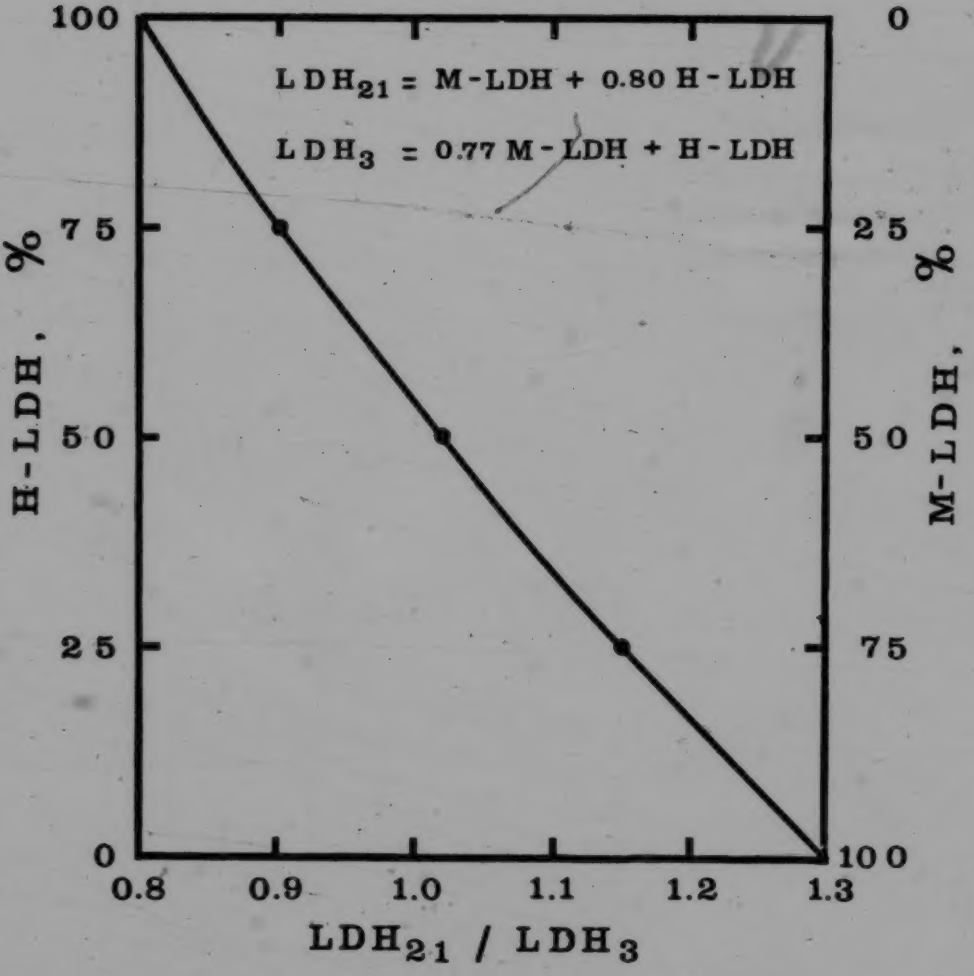

FIGURE 4. PERCENTAGE OF H-LDH AND M-LDH FROM THE LDH_{21}/LDH_3 RATIO. THE LINE HAS BEEN DRAWN BY SUBSTITUTING THE % OF M-LDH AND H-LDH IN THE INSERTED EQUATIONS WHICH HAVE BEEN OBTAINED FROM TABLE 21 AND FIGURE 6 OF APPENDIX A

Final spectrophotometric conditions of the reagents in the cuvets were pH 7.4, 0.067 M Phosphate buffer (Sorensen), 1.5×10^{-4} M NADH and 21×10^{-4} or 3×10^{-4} M pyruvate.

Adenosine triphosphate and phosphorylcreatine were measured in the same assay according to Lamprecht and Stein (1963b) and Lowry and Passonneau (1972, p. 151). The reactions, as for CPK, are as follows:

$$\text{PC} + \text{ADP} \xrightleftharpoons{\text{CPK}} \text{creatine} + \text{ATP} \qquad (1)$$

$$\text{glucose} + \text{ATP} \xrightleftharpoons{\text{HK}} \text{G6P} + \text{ADP} \qquad (2)$$

$$\text{G6P} + \text{NADP} \xrightleftharpoons{\text{G6P-DH}} \text{6-phosphogluconate} + \text{NADPH} + \text{H}^{+} \qquad (3)$$

ATP was measured using Calbiochem Kits (ATP Stat-Pack No. 869206) which were modified for PC by adding other reagents to reach the final concentrations suggested by Lamprecht and Stein (1963b). This modification yielded identical results to the conventional approach but saved considerable time.

Statistical Methods

Assays were all done in duplicate and computation carried out on the means. Suspect values were rejected on a common sense basis. Calculated t for the rejected values were higher than the 1.9 and even the 2.44 rejection criteria ($\alpha = 0.05$ and 0.025 respectively for $n = 7$). Percentages of missing data appear in Appendix F. Data were analysed from two points of view: differences between training groups and differences between organs (or tissues). Group and organ means and standard deviations were calculated for all the dependant variables.

Two way analyses of variance (Winer, 1971, pp. 245-248) were made between group differences and between organ differences. Since the variance differed very much between some variables, and since there were no significant interactions between training groups and organs, and since the differences between organs were much larger than the ones between training groups (Appendix F), the two way analyses of variance were merely used to assess the general pattern between training groups. To assure more specific analyses, one way layouts (Winer, 1971, pp. 210-219) were done on each variable and each organ to assess training group differences and on each variable with combined data from all groups to assess organ differences.

For comparison between pairs of means, Scheffé's contrasts (Scheffé, 1959, pp. 66-67; Winer, 1971, pp. 198-201) were computed for α equal to 0.05 when the F ratios of the analysis of variance were significant ($p \leq 0.05$). Although less powerful than other a posteriori tests, Scheffé's method was used because it is clearly the most conservative with respect to type I error (Scheffé, 1959; Winer, 1971). Scheffé's procedure does not require equal n and is less sensitive to violations of normality and homogeneity of variance assumptions than Tukey's procedure, the only other comparable test with respect to Type I error (Myers, 1966, pp. 333-336). All statistics were computed with SCIRU (Service de consultation informatique pour la recherche universitaire), one of the services at the Université de Montréal.

CHAPTER IV

RESULTS

The raw data for the training groups are listed in Appendix C. The original statistics on dependent variables which showed significant differences ($P < 0.05$) between training groups appear in Appendices D, E and F. Training group comparisons for each dependent variable: means, standard deviations, ANOVA F ratios and Scheffé's contrasts, are found in Tables 4 to 17. The group body weight growth curves are plotted in Figure 5 from data found in Table 22 (see Appendix B).

Significant ANOVA F ratios ($P < 0.05$) were obtained between training groups for absolute body weight, relative organ weight, ATP and CPK. All other variables (PC, ATP + PC, AK, LDH_{21}, LDH_3, LDH_{21}/LDH_3, M-LDH, H-LDH and Total LDH) showed no significant trend with one way ANOVA. However, two way ANOVA revealed significant differences between training groups for these parameters (Appendix F).

The details of the aforementioned results are presented in the two following sections: "The Effect of Chronic Exercise" and "Organ Comparison".

The Effects of Chronic Exercise

Body Weight

The body weights obtained for the rats at each week during the study were averaged by group (Table 22, Appendix B). Weight progress is depicted graphically in Figure 5. During the initial exercise program, body weights were equal and progressed at the same rate for all groups. During the formal training period however, the increases in weight of the training groups progressed at a much slower rate than Sgr ($P < 0.005$, Table 4). Scheffé's contrasts were significant between Sgr (625 g) and the training groups (Cgr: 534 and Igr: 534 g) but not between the training groups themselves. Growth rate slowly declined with age and started to plateau by 25 weeks of age.

Organ Weights

Both absolute and relative weights of the organs were recorded at the time of sacrifice. There were no significant differences in absolute organ weights between the groups (Table 5). Relative organ weights (except liver) of the trained animals were heavier than those of Sgr (Table 6). However, F ratios were significant only for the tibialis anterior, the plantaris, the gastrocnemius medialis and the heart. Furthermore, Scheffé's contrasts indicated that only the plantaris muscles of Cgr were heavier than those of Sgr.

TABLE 4 Final Body Weight for the Different Training Groups: Means, Standard Deviations, ANOVA F ratio and Scheffé's Contrasts.

Sgr[ab] g	Cgr[ab] g	Igr[ab] g	F	P <	Contrasts ($P < .05$)
625 70	534 42	534 44	7.698	0.005	Cgr < Sgr Igr < Sgr

a. abbreviations: see Table 1.
b. n = 8

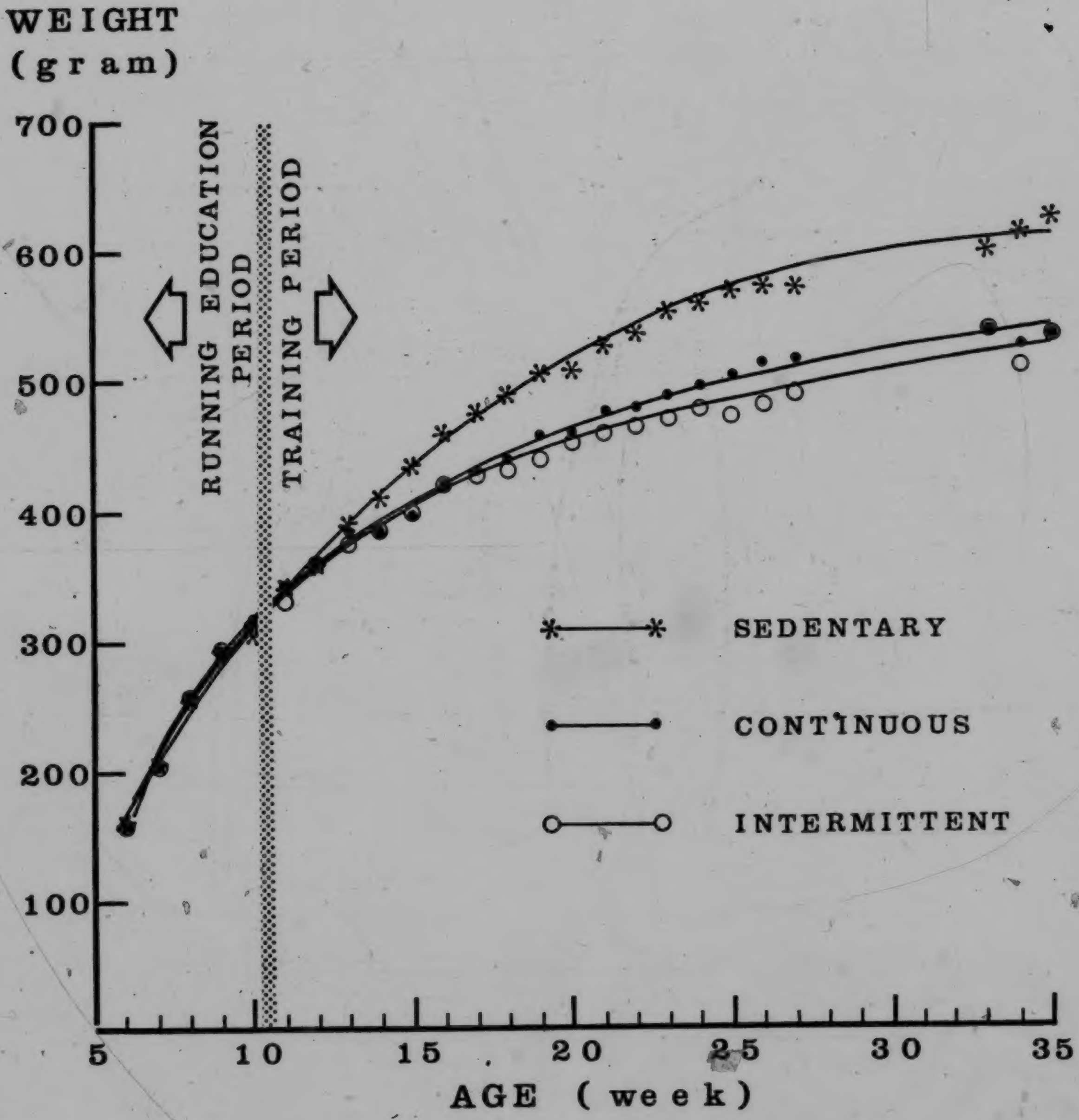

FIGURE 5. RAT BODY WEIGHT GAIN WITH AGE AND TRAINING (DRAWN FROM TABLE 22, APPENDIX B)

TABLE 5 Absolute Weight of the Organs in the Different Training Groups: Means, Standard Deviations, and ANOVA F Ratios.

Organs[a]	Sgr[ab] mg	Cgr[ab] mg	Igr[ab] mg	F	P<
TA	998 124	1003 47	968 87	0.345	ns
P	562 58	582 53	551 58	0.591	ns
GM	1194 109	1244 100	1222 97	0.491	ns
GL	1440 173	1405 94	1450 189	0.176	ns
S	250 48	258 27	251[c] 31	0.116	ns
H	1496[d] 153	1471[d] 179	1499[c] 77	0.074	ns
L	15997 1364	13957 1544	12389 4852	2.827	ns

a. abbreviations: see Table 1.
b. n = 8
c. n = 7
d. n = 6

TABLE 6 Relative Weight of Organs in the Different Training Groups: Means, Standard Deviations, ANOVA F Ratios and Scheffé's Contrasts.

Organs[a]	Sgr[ab] mg/g	Cgr[ab] mg/g	Igr[ab] mg/g	F	P<	Contrasts (P < .05)
TA	1.62 0.07	1.88 0.10	1.82 0.22	3.635	0.05	Cgr & Igr > Sgr
P	0.91 0.17	1.09 0.05	1.04 0.14	3.783	0.05	Cgr > Sgr
GM	1.94 0.29	2.33 0.09	2.29 0.18	9.296	0.01	Cgr > Sgr Igr > Sgr
GL	2.34 0.47	2.64 0.14	2.72 0.35	2.584	ns	
S	0.41 0.10	0.48 0.03	0.47[c] 0.06	2.508	ns	
H	2.32[d] 0.20	2.73[d] 0.33	2.81[c] 0.27	5.814	0.05	Igr > Sgr
L	25.94 4.30	26.13 2.04	25.93 3.45	0.008	ns	

a. abbreviations: See Table 1.
b. n = 8
c. n = 7
d. n = 6

Adenosine Triphosphate and Phosphorylcreatine

Resting concentrations of ATP and PC as well as ATP + PC are reported for each group and each organ in Tables 7 to 9. Only ATP concentrations showed significant group differences. Scheffé's contrasts further revealed that for the tibialis anterior, ATP was lower in Igr compared to either Cgr or Sgr. For the gastrocnemius medialis and lateralis, ATP was lower in Igr compared to Cgr. Finally, the soleus of Cgr had higher ATP values than the two other groups. Therefore, the general tendency was to have a higher ATP concentration in Cgr, followed by Sgr and then Igr. This was confirmed by analysis of variance and contrasts run on the combined data from all organs (Appendix F).

Creatinephosphokinase and Adenylate Kinase

CPK and AK activities and training group comparisons are reported in Tables 10 and 11. None of the organs showed significant difference between training groups for AK. On the other hand, combined data from all organs indicated that CPK was higher in Sgr compared to the trained groups (Appendix F).

Lactate Dehydrogenase

LDH related variables for group comparisons are reported in Tables 12 to 17. Two way analysis of variance (Appendix F) revealed that LDH_{21}, LDH_3, M-LDH and total LDH were higher in all organs of Sgr compared to the trained groups. One way analysis of variance showed less significant differences between training groups. LDH_{21} and LDH_3 activities were higher in the gastrocnemius lateralis of Sgr compared to Cgr whereas M-LDH was higher in the heart of Cgr than in Igr. No significant difference between groups characterized the LDH_{21}/LDH_3 ratio (or the percentage of M-LDH and H-LDH) and the H-LDH activity.

Organ Comparison

Organ means, standard deviations and ANOVA F ratios from combined group data* for each dependant variable appear in Table 18. Significant Scheffé's contrasts are identified in Table 19. In general, the organs fell into four distinct categories: liver, heart, soleus and the other skeletal muscles.

Adenosine Triphosphate and Phosphorylcreatine (Tables 18 and 19)

PC was the lowest in the heart (5.5 mmoles/kg) slightly higher in the soleus (9.0 mmoles/kg) and much higher in other skeletal muscles (14.2 - 18.3 mmoles/kg). With a few exceptions, ATP and ATP + PC were characterized by a similar pattern. For instance, ATP was higher in the heart (4.2 mmoles/kg) than in the soleus (3.4 mmoles/kg).

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* Since the differences were much larger between organs than between groups, group data were pooled.

TABLE 7 ATP for Each Organ in the Different Training Groups: Means, Standard Deviations, ANOVA F Ratios and Scheffé's Contrasts.

Organs[a]	Sgr[ab] mmoles/kg wet weight	Cgr[ab] mmoles/kg wet weight	Igr[ab] mmoles/kg wet weight	F	P<	Contrasts ($P < .05$)
TA	5.8 0.5	5.8 0.9	4.9 0.4	5.875	0.01	Igr < Sgr Igr < Cgr
P	5.1 0.7	5.3[c] 0.8	4.6 0.5	2.324	ns	
GM	5.2 1.1	5.8 0.9	4.5 0.8	3.775	0.05	Igr < Cgr
GL	4.8 0.5	5.3 1.0	4.3 0.3	4.312	0.05	Igr < Cgr
S	3.3 0.2	3.9 0.5	3.2 0.5	6.924	0.01	Sgr < Cgr Igr < Cgr
H	4.0 0.4	4.5 0.5	4.3 0.4	2.402	ns	

a. abbreviations: see Table 1
b. n = 8
c. n = 7

TABLE 8 PC for Each Organ in the Different Training Groups: Means, Standard Deviations and ANOVA F Ratios.

Organs [a]	Sgr[ab] mmoles/kg wet weight	Cgr[ab] mmoles/kg wet weight	Igr[ab] mmoles/kg wet weight	F	P
TA	18.9 4.2	18.9 2.0	16.9[c] 2.4	0.979	ns
P	15.9 6.8	13.2 2.6	15.1[c] 3.3	0.617	ns
GM	15.5 2.5	16.8 3.6	16.7[c] 3.5	0.365	ns
GL	14.7[c] 2.6	14.4 2.7	13.4[c] 3.0	0.385	ns
S	8.9 1.8	9.7 3.2	8.0[d] 1.0	0.830	ns
H	5.3[c] 1.2	5.9[c] 0.7	5.2[c] 1.8	0.533	ns

a. abbreviations: see Table 1
b. n = 7
c. n = 6
d. n = 5

TABLE 9 ATP + PC for Each Organ in the Different Training Groups: Means, Standard Deviations and ANOVA F Ratios.

Organs[a]	Sgr[a,b] mmoles/kg wet weight	Cgr[a,b] mmoles/kg wet weight	Igr[a,b] mmoles/kg wet weight	F	P
TA	24.7 4.0	24.7 1.5	21.4[c] 2.4	2.825	ns
P	21.1 6.6	18.2 3.2	19.8[c] 3.6	0.629	ns
GM	20.6 2.9	22.5 4.2	21.3[c] 4.3	0.440	ns
GL	19.7[c] 2.4	19.7 3.3	17.6[c] 3.3	0.949	ns
S	12.2 1.9	13.7 3.4	11.0[d] 1.5	1.766	ns
H	9.3[c] 1.5	10.0[c] 1.5	9.6[c] 2.0	0.318	ns

[a] abbreviations: see Table 1

[b] n = 7

[c] n = 6

[d] n = 5

TABLE 10 CPK for Each Organ in the Different Training Groups: Means, Standard Deviations, ANOVA F Ratios and Scheffé's Contrasts.

Organ^{a}	Sgr^{ab} IU/g wet weight	Cgr^{ab} IU/g wet weight	Igr^{ab} IU/g wet weight	F	P<	Contrasts (P < .05)
TA	2870^{d}	2540^{c}	2420	2.463	ns	
	400	410	330			
P	2780	2440^{c}	2230	3.757	0.05	Igr < Sgr
	340	430	440			
GM	2960	2340^{c}	2350	4.492	0.05	Igr & Cgr < Sgr
	550	440	410			
GL	2770	1960	1900	11.723		Cgr < Sgr
	460	350	390			Igr < Sgr
S	1040	1060	1140^{c}	0.737	ns	
	220	170	90			
H	810	910	890	0.907	ns	
	160	180	120			
L	10.2^{c}	17.4	14.7	1.358	ns	
	2.5	9.4	10.7			

a. Abbreviations: see Table 1
b. n = 8
c. n = 7
d. n = 6

TABLE 11 AK for Each Organ in the Different Training Groups: Means, Standard Deviations and ANOVA F Ratios.

Organ^{a}	Sgr^{ab} IU/g wet weight	Cgr^{ab} IU/g wet weight	Igr^{ab} IU/g wet weight	F	P <
TA	133^{d} 61	122 71	132^{c} 36	0.082	ns
P	129^{d} 62	124 72	125^{c} 39	0.012	ns
GM	121^{d} 53	122 74	126^{c} 44	0.013	ns
GL	118^{d} 52	96^{c} 62	113^{c} 38	0.389	ns
S	62^{d} 25	52^{c} 27	65^{d} 28	0.439	ns
H	61^{d} 18	65 32	71^{c} 24	0.281	ns
L	20^{d} 3	19^{c} 4	20^{c} 6	0.066	ns

a. abbreviations: see Table 1.
b. n = 8
c. n = 7
d. n = 6

TABLE 12 LDH_{21} for Each Organ in the Different Training Groups: Means, Standard Deviations, ANOVA F Ratios and Scheffé's Contrasts.

Organ[a]	Sgr[ab] IU/g wet weight	Cgr[ab] IU/g wet weight	Igr[ab] IU/g wet weight	F	P <	Contrasts (P < .05)
TA	587 164	452 123	469 136	2.161	ns	
P	599[c] 111	500 152	503 91	1.567	ns	
GM	482[d] 105	440 110	442[c] 109	0.306	ns	
GL	505 104	362 94	408[c] 121	3.752	0.05	Cgr < Sgr
S	131 40	138[e] 57	117[c] 25	0.459	ns	
H	280[d] 73	289[c] 129	270 66	0.074	ns	
L	325[c] 79	246[d] 90	300[d] 58	1.727	ns	

a. abbreviations: see Table 1.
b. n = 8
c. n = 7
d. n = 6
e. n = 5

TABLE 13 LDH_3 for Each Organ in the Different Training Groups: Means, Standard Deviations, ANOVA F Ratios and Scheffé's Contrasts.

Organ[a]	Sgr[ab] IU/g wet wright	Cgr[ab] IU/g wet weight	Igr[ab] IU/g wet weight	F	P	Contrasts ($P < .05$)
TA	486	364	387	2.207	ns	
	142	108	120			
P	510[c]	420	425	1.630	ns	
	91	138	91			
GM	423[d]	354	387[c]	0.976	ns	
	83	95	93			
GL	431	315	351[c]	3.667	0.05	Cgr < Sgr
	91	93	91			
S	143	151[e]	121[c]	0.879	ns	
	38	59	33			
H	327[d]	322[c]	334	0.019	ns	
	94	149	91			
L	279[c]	207[d]	258[d]	1.384	ns	
	85	82	68			

a. abbreviations: see Table 1.
b. n = 8
c. n = 7
d. n = 6
e. n = 5

TABLE 14 LDH$_{21}$/LDH$_3$ for Each Organ in the Different Training Groups: Means, Standard Deviations, M-LDH (%) and ANOVA F Ratios.

Organ^{a}	Sgr^{ab}	Cgr^{ab}	Igr^{ab}	F	P
TA	1.21	1.26	1.22	0.445	ns
	0.09	0.11	0.11		
	85.3	91.1	85.6		
P	1.17^{c}	1.21	1.20	0.259	ns
	0.12	0.10	0.09		
	79.6	82.7	81.4		
GM	1.14^{d}	1.25	1.15^{c}	2.182	ns
	0.10	0.11	0.12		
	73.5	90.9	74.2		
GL	1.17	1.17	1.16^{c}	0.045	ns
	0.08	0.11	0.12		
	79.2	75.2	77.8		
S	0.92	0.93^{e}	0.98^{c}	0.538	ns
	0.13	0.13	0.09		
	28.7	28.3	39.6		
H	0.87^{d}	0.90^{c}	0.82	3.391	ns
	0.08	0.04	0.06		
	14.3	23.6	2.5		
L	1.18^{c}	1.20^{d}	1.18^{d}	0.095	ns
	0.10	0.06	0.10		
	77.8	82.3	78.0		

a. abbreviations: see Table 1.
b. n = 8
c. n = 7
d. n = 6
e. n = 5

TABLE 15 M-LDH for Each Organ in the Different Training Groups: Means, Standard Deviations, ANOVA F Ratios and Scheffé's Contrasts.

Organ^a	Sgr^{ab} IU/g wet weight	Cgr^{ab} IU/g wet weight	Igr^{ab} IU/g wet weight	F	P	Contrasts ($P < .05$)
TA	516 164	419 126	415 136	1.284	ns	
P	497^c 171	428 123	426 80	0.731	ns	
GM	374^d 143	407 119	346^c 140	0.398	ns	
GL	417 135	287 83	332^c 149	2.258	ns	
S	44 54	41^e 70	52^c 20	0.092	ns	
H	48^d 53	80^c 35	8 58	3.884	0.05	Igr < Cgr
L	264^c 47	210^d 68	245^d 39	1.769	ns	

a. abbreviations: see Table 1
b. n = 8
c. n = 7
d. n = 6
e. n = 5

TABLE 16 H-LDH for Each Organ in the Different Training Groups: Means, Standard Deviations and ANOVA F Ratios.

Organ[a]	Sgr[ab] IU/g wet weight	Cgr[ab] IU/g wet weight	Igr[ab] IU/g wet weight	F	P
TA	89 102	41 89	68 97	0.505	ns
P	127[c] 120	90 78	97 86	0.323	ns
GM	135[d] 99	40 81	120[c] 106	2.082	ns
GL	110 82	94 81	88[c] 80	0.157	ns
S	109 50	113[e] 65	80[c] 40	0.828	ns
H	290[d] 113	253[c] 116	327 118	0.760	ns
L	68[c] 74	45[d] 40	69[d] 72	0.271	ns

a. abbreviations: see Table 1.
b. n = 8
c. n = 7
d. n = 6
e. n = 5

TABLE 17 Total LDH for Each Organ in the Different Training Groups: Means, Standard Deviations and ANOVA F Ratios.

Organ[a]	Sgr[ab] IU/g wet weight	Cgr[ab] IU/g wet weight	Igr[ab] IU/g wet weight	F	P
TA	605 170	460 128	477 145	2.277	ns
P	624[c] 105	512 165	498 65	2.462	ns
GM	509[d] 103	448 114	466[c] 111	0.536	ns
GL	516 124	381 104	420[c] 116	2.916	ns
S	147 40	154[e] 59	133[c] 31	0.392	ns
H	338[d] 92	355[c] 154	335 86	0.060	ns
L	340[c] 91	255[d] 97	314[d] 70	1.578	ns

a. abbreviations: see Table 1.
b. n = 8
c. n = 7
d. n = 6
e. n = 5

TABLE 18 Weights, Metabolite Concentrations and Enzyme Activities in the Different Organs: Means and Standard Deviations from Combined Data for All Training Groups.

Variables[ab]	TA	P	GM	GL	S	H	L
W_{abs} (mg)	990 89	565 57	1220 100	1432 152	253 35	1489 132	14114 3276
W_{rel} (mg/g)	1.77 0.23	1.01 0.15	2.19 0.27	2.57 0.37	0.45 0.08	2.63 0.34	26.00 3.24
ATP mmole/kg	5.5 0.8	5.0 0.7	5.2 1.0	4.8 0.8	3.5 0.5	4.2 0.4	
PC mmole/kg	18.3 3.0	14.7 4.6	16.3 3.1	14.2 2.7	9.0 2.3	5.5 1.4	
ATP + PC mmole/kg	23.7 3.0	19.7 4.7	21.5 3.7	19.0 3.0	12.4 2.6	9.7 1.6	
CPK (IU/g)	2590 41	2490 45	2560 54	2210 56	1080 17	870 16	14.2 8.7
AK (IU/g)	128 56	126 57	123 56	108 50	59 26	66 25	20 4
LDH_{21} (IU/g)	503 149	531 124	452 105	426 119	128 39	279 89	292 80
LDH_3 (IU/g)	412 130	449 110	385 91	366 97	137 42	328 109	250 81
$\frac{LDH_{21}}{LDH_3}$	1.23 0.10	1.23 0.10	1.18 0.12	1.16 0.10	0.94 0.11	0.86 0.07	1.19 0.08
M-LDH (IU/g) %[c]	450 144 87.2	448 126 81.2	377 129 80.0	350 131 77.6	46 48 32.0	44 57 12.9	241 55 79.0
H-LDH (IU/g) %[c]	66 94 12.8	104 92 18.8	94 100 20.0	99 78 20.4	100 50 68.0	292 115 87.1	61 62 21.0
TOTAL LDH (IU/g)	514 157	541 127	471 108	440 124	144 41	343 109	305 90

a. All ANOVA F ratios for organ comparison were significant ($P < 0.001$).

b. The number of samples per cell was between 19 and 24.

c. Calculated from LDH_{21}/LDH_3 and Figure 4 (Methods and Procedures).

TABLE 19 Weights, Metabolite Concentrations and Enzyme Activities in the Different Organs: Scheffé's Contrasts from Combined Data for All Training Groups.

Contrasts*	W_{abs}	W_{rel}	ATP	PC	ATP + PC	CPK	AK	LDH_{21}	LDH_3	$\frac{LDH_{21}}{LDH_3}$	M-LDH	H-LDH	TOTAL LDH
P-TA	ns	ns	ns	-	-	ns	ns	ns	ns	ns	ns	ns	ns
GM-TA	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
GM-P	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
GL-TA	ns	ns	ns	-	-	ns	ns	ns	ns	ns	ns	ns	ns
GL-P	ns	+	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
GL-GM	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
S-TA	ns	ns	-	-	-	-	-	-	-	-	-	ns	-
S-P	ns	ns	-	-	-	-	-	-	-	-	-	ns	-
S-GM	ns	-	-	-	-	-	-	-	-	-	-	ns	-
S-GL	ns	-	-	-	-	-	ns	-	-	-	-	ns	-
H-TA	ns	ns	-	-	-	-	-	-	ns	-	-	+	-
H-P	ns	+	ns	-	-	-	-	-	-	-	-	+	-
H-GM	ns	ns	-	-	-	-	-	-	ns	-	-	+	-
H-GL	ns	ns	ns	-	-	-	ns	-	ns	-	-	+	ns
H-S	ns	+	+	-	ns	ns	ns	ns	+	ns	ns	+	+
L-TA	+	+				-	-	-	-	ns	-	ns	-
L-P	+	+				-	-	-	-	ns	-	ns	-
L-GM	+	+				-	-	-	-	ns	-	ns	-
L-GL	+	+				-	-	-	-	ns	ns	ns	-
L-S	+	+				-	ns	+	ns	+	+	ns	+
L-H	+	+				-	ns	ns	ns	+	+	-	ns

* ns: not significant ($P < 0.05$); "+" is a positive difference; "-" is a negative difference.

Creatine Phosphokinase and Adenylate Kinase (Tables 18 and 19)

CPK was much lower in the liver than in any other organ. Heart and soleus CPK were not different but had only half the values found in the other muscles. AK was twice as active in the tibialis anterior, the plantaris and the gastrocnemius as in the soleus, the heart and the liver.

Lactate Dehydrogenase (Tables 18 and 19)

LDH_{21} was found to be lower in the soleus than in the heart and the liver and highest in the other muscles. For LDH_3, the pattern was low activity in the soleus, slightly higher activity in the liver and highest activity in the other muscles including the heart.

The percentage of M-LDH and of H-LDH from Total LDH as indicated by LDH_{21}/LDH_3 ratios was lower in the heart and the soleus than in the liver and other skeletal muscles. M-LDH activity was found to be lowest in the heart and the soleus, much higher in the liver and highest in the other muscles. H-LDH was three times more active in the heart than in any other organ. Total LDH was lowest in the soleus, two times higher in the heart and liver and approximately three times higher in the other skeletal muscles.

CHAPTER V

DISCUSSION

The discussion will be divided into the following two sections: differences between selected muscles and liver and the chronic effects of exercise. For each of the selected parameters, differences between sampled muscles will first be analysed in order to compare the data in the present study with literature values. This comparison between sampled tissues will result in a classification that should make easier the following discussion of the chronic effects of exercise on the selected parameters.

Differences Between Selected Muscles and Liver (Tables 18 and 19)

Since the enzyme and metabolite concentrations usually vary from one fiber type to another, it is appropriate to identify the fiber composition of the muscles selected for this study (Table 20). According to the classifications used by Ariano et al. (1973), Barnard et al. (1970a), Edgerton et al. (1969 and 1975) and Peter (1970), it can be seen that except for the soleus which is predominantly composed of SO fibers, other skeletal muscles are mostly composed of FT fibers with equal proportions of FG and FOG fibers. The soleus and the other skeletal muscles will be identified as the ST soleus and the FT muscles in the following discussion.

TABLE 20 Literature Values of Fiber Type Composition of Selected Rat Skeletal Muscles.

Muscle	Reference	Fiber Types* (%)		
		SO	FOG	FG
TIBIALIS ANTERIOR	Ariano et al. (1973)	2	66	32
	Close (1972)	15-20	40	40-45
PLANTARIS				
Total	Ariano et al. (1973)	6	53	41
Superficial	Edgerton et al. (1969)	15	10	75
Deep	Edgerton et al. (1969)	20	25	55
GASTROCNEMIUS				
Total	Schmalbruch et al. (1975)	15	50	30
Medialis	Ariano et al. (1973)	4	38	58
Lateralis	Ariano et al. (1973)	5	37	58
"	Muller (1974)** Untrained	9	19-53	38-71
	Trained	12	34-50	38-55
SOLEUS	Ariano et al. (1973)	84	14	0
	Baldwin et al. (1972)	96	4	0
	Close (1972)	85-90	10-15	0
	Edgerton et al. (1969)	80	20	0
	Schmalbruch et al.(1975)	94	6	0
	Muller (1974)** Untrained	86	13	0
	Trained	96.3	2.7	0

* Slow twitch oxidative, fast twitch high oxidative glycolytic and fast twitch glycolytic respectively.

** Estimation from another classification.

Before considering the tissue or muscle differences for each of the studied parameters, it is worthwhile to note that the enzyme ratios (i.e. LDH/CPK, AK/CPK and LDH/AK) found in this study agree with those reported by Pette (1975).

Lactate Dehydrogenase in Various Tissues

As the assay techniques as well as the enzyme activity units vary from one study to another, activity ratios are more useful than absolute activity in comparing the validity of the present results with previously published findings. As a whole, this study (Tables 18 and 19) revealed that Total LDH activity was the lowest in the soleus (144 IU/g), 2X higher in the heart (343 IU/g) and the liver (305 IU/G), and approximately 3X higher in the other skeletal muscles (440 to 541 IU/g). Karlsson _et al_. (1975) and Sjodin _et al_. (1976b) reported that total LDH is proportional to the % of FT fibers which are mostly composed of M-LDH, as was also found in the present study. However, heart was not studied by these investigators and this muscle does not seem to follow a similar trend. The method for calculating total LDH in the present study may partially explain this difference. Total activity is the summation of M-LDH and H-LDH activity calculated from LDH activity at low and high pyruvate concentrations which were found to be optimal for H-LDH and M-LDH respectively (see Chapter III on Methods and Procedures). In other studies (Karlsson _et al_., 1974b; Peter _et al_., 1971; Sjodin, 1976a), total LDH was measured at one pyruvate concentration. It is possible that M-LDH has been favored when compared to H-LDH. To illustrate this rationale, a comparison of the LDH values for the heart and the gastrocnemius lateralis (Tables 18 and 19) can be made. For LDH at high pyruvate concentration, the gastrocnemius (426 IU/g) is much more active than the heart (279 IU/g), which is consistent with the proposed lower LDH activity in ST muscles. However, for LDH at low pyruvate concentrations, the heart (328 IU/g) is similar to the gastrocnemius (366 IU/g); also, Total LDH for the heart 343 IU/g) and the gastrocnemius (440 IU/g) was not significantly different. Thus the ST % alone appears insufficient to explain the level of Total LDH activity. It is speculated that the LDH distribution pattern might be, partially at least, explained by the actual state and potential of oxidative activity of the muscles as well by their ST %. For example, FOG portions of muscles usually have a lower LDH activity than FG portions, although they have the same percentage of FT (or ST) fibers (Baldwin et al., 1973; Peter et al., 1971; York et al., 1974). The LDH activity of FOG and FG muscle portions behaves reciprocally with the muscle's oxidative capacity, as measured by different markers such as citrate synthase, carnitine palmityltransferase, cytochrome a and cytochrome c activities as well as the pyruvate - 2 - ^{14}C and palmitate - U - ^{14}C oxidations (Baldwin et al., 1972a, 1977a and b; Holloszy et al., 1975; Peter et al., 1971). Such a relationship is also supported by the two following facts: firstly, muscle samples from endurance-trained athletes have been shown to possess higher oxidative capacity and lower LDH activity compared to muscle with the same FT/ST fiber ratio found in sedentary subjects (Karlsson et al., 1975; Sjodin et al., 1976b) and secondly, the heart, with the highest oxidative capacity (Baldwin et al., 1977a; Holloszy et al., 1975; Peter et al., 1971) also has lower LDH activity than FT muscles (Tables 18 and 19), and even FOG muscle portions (Peter et al., 1971). Since the oxidative capacity of the soleus is intermediary between the FG and FOG muscle portions, and much lower than the heart (Baldwin et al., 1972a, 1977a and b; Holloszy et al., 1975; Peter et al., 1971), the oxidative capacity of the muscle cannot explain the low LDH activity of the ST soleus found in this study and confirmed by others (Baldwin et al., 1973; Peter et al., 1971). Thus, both the oxidative capacity and the percentage of ST fibers appears to regulate the LDH activity levels. It is possible that at high intensity, work is carried out more by the FT muscles or fibers and less by the ST muscles or fibers, which would in turn, explain the lowest LDH activity of the soleus. In the other cases, and as long as the oxidative potential is not reached, the energy demand must be met either by the aerobic or the anaerobic metabolism and the higher the former, the lower the latter.

Besides the small differences that have been described above between Total LDH and LDH measured at one pyruvate concentration, the two values generally agree. From this point of view, organ to organ ratios are similar to those reported in the literature for the rat and guinea pig (Baldwin et al., 1973; Doty et al., 1971; Gollnick et al., 1961, 1967; Hickson et al., 1976; Peter et al., 1971; Staudte et al., 1973; York et al., 1974, 1975, 1976), and human skeletal muscles (Gollnick et al., 1974; Karlsson et al., 1975; Sjodin et al., 1976a and b). There does not seem to be much difference between man and rodents although rabbit livers contain 40% M-LDH compared to 95% and more for man and rat (Fine et al., 1963).

M-LDH% values were similar for the soleus and the heart ($\bar{X} = 23\%$) and much higher in the liver and skeletal muscles ($\bar{X} = 84\%$). This finding agrees with other reported values for the rat, guinea pig and human (Fine et al., 1963; Hirota et al., 1976; Peter et al., 1971; Plageman et al., 1960a; Sjodin et al., 1976a and b; Thorling and Jenson, 1966; York et al., 1974, 1975, 1976). Thus, these results support the effectiveness of the assay technique used in this study.

Creatine Phosphokinase and Adenylate Kinase in Various Tissues

As shown in Tables 18 and 19, CPK and AK were generally much higher in FT skeletal muscles (2500 and 120 IU/g respectively) than in the ST soleus (1080 and 59 IU/g), the heart (870 and 66 IU/g) and the liver (14 and 19 IU/g. These findings are in accord with the muscle to muscle activity ratios for rat and human tissue reported by others (Bernstein et al., 1973; Dieter, 1970; Jacob et al., 1964;

Haralambie, 1972 and 1973; Kleine and Chlond, 1967; Newsholme and Start, 1973; Oliver, 1955; Pette, 1971; Staudte et al., 1973). In general, CPK and AK are higher in FT muscle as compared to ST muscle. Such a specific pattern might have some implications for the effects of chronic exercise on these enzymes.

Adenosine Triphosphate and Phosphorylcreatine in Various Tissues

ATP and PC values in the present study showed similar patterns of difference from one tissue to the other and are as high as the ones reported in the literature for rat and human (Degenring et al., 1975; Fawaz et al., 1962; Harris et al., 1974; Hultman et al., 1967; Karlsson, 1971a; Leunissen and Piatnek-Leunissen, 1968; Keul et al., 1972, p. 32; Pool et al., 1976; Pourel, 1968; Rabinowitz and Zak, 1975; Swynghedauw et al., 1960). Thus, the difficult problem of rapid tissue fixation (particularly in the heart) seems to have been avoided. As shown in Tables 18 and 19, PC is twice as high in FT skeletal muscles (14 to 18 mmoles/kg) as in the ST soleus (9 mmoles/kg) and the heart (5.5 mmoles/kg).

In general, it can be stated that the glycolytic (LDH) and high-energy phosphate transferase enzymes (CPK and AK) as well as the ATP and PC stores are higher in the most anaerobic tissues.

The Chronic Effects of Exercise

Before discussing the effects of chronic exercise on the different parameters, some general aspects will be analysed. Firstly, the training effects reported in this study cannot be qualitatively characterized since there were no growth control groups in the study. In other words, although the trained groups might have a higher enzyme activity than the sedentary groups, which is a real difference, it cannot be stated that training has increased the enzyme activity because it may have prevented a possible decrease in activity with aging. Nevertheless, it seems appropriate to discuss the differences between the experimental groups.

Secondly, the fact that training was slightly reduced at the end of the training period might have a reversing effect on the parameters studied. However, as judged by the exhausted state of the rats after each training session, it was felt that the rats were still overloaded. Barnard and Peter (1971), who also had to reduce the training load of their animals, reported that cytochrome a and c continued to increase after this reduced work load. Thus, reduced work load might only have slowed down the improvement rate or maintained the activity level. The reasons for such a decrease in training load are not understood at the present time.

Body and Organ Weights (Tables 4 and 6)

As found in other studies (Baldwin et al., 1972; Crews et al., 1969; Dowell et al., 1976; Edington and Cosmos, 1972; Gollnick et al., 1961 and 1967; Holloszy, 1967; Hubbard et al., 1974; Oscai et al., 1971; Pattengale and Holloszy, 1967; Rogozskin, 1976; Ruhling et al., 1973), endurance training reduced by 15% the usual weight gain found in sedentary male rats (Table 4 and Figure 5). This adaptation supports the effectiveness of the endurance training regimen used in the present study. Sprint training has been shown to inhibit rat body weight gain to a similar extent. Staudte et al. (1973) have reported a 10% decrease in rat body weight after 21 days of sprint training and Rhuling et al. (1973) observed a 6-8% decrease in rat body weight after sprint training as compared to 14% and 4% after endurance running and swimming respectively.

The week by week rate of weight gain for trained and sedentary rats is depicted in Figure 5. Some points fell off the curve at one time or another. These variations appear to be normal as judged by other published curves (Booth, 1972; Freminet et al., 1975; Mayer et al., 1954; Muller, 1975) and surely reflect the precision and sensitivity of the mechanism of correction by successive compensation (or the long term regulation of the energy balance) discovered by André Mayer (Mayer, 1968).

Other possible explanations for the week by week abrupt changes in body weight are 1) the irregularity in the weighing period time, 2) reduced population of the experimental group due to occasional death of a few animals (Table 22, Appendix B), 3) uncontrolled variations in diet or other living conditions of the animals under the care of the animal service of the University and 4) variations in the training load that were continuously adjusted to the estimated exhaustion levels of the rats. For these reasons and because the objective was to study the main effect of the exercise regimen on body weight, a curve of best fit was utilized. The exact causes of the abrupt changes remain to be however elucidated.

Organ weights were also measured to see if training induced hypertrophy. The absolute weights of the organs were similar in the three experimental groups (Table 5). However, the reduced body weight of both training groups may have masked organ hypertrophy since the relative weights of the organs (e.g. organ weight in mg/body weight in g) were higher in the heart and skeletal muscles but not in the liver of the trained groups (Table 6). Heart hypertrophy after endurance running and more particularly after endurance swimming training in rats is well documented (Baldwin et al., 1977a; Codini et al., 1977; Dowell et al., 1976; Gollnick et al., 1967; Hepp et al., 1974; Oscai et al., 1971; Penpargkul and Scheuer, 1970; Walpurger and Anger, 1970). Heart hypertrophy has also been reported after sprint training of the rat (Baldwin et al., 1977a). In skeletal muscle, endurance training does not seem to induce hypertrophy (Baldwin et al., 1972 and 1977b; Holloszy, 1967; Oscai et al., 1971; Pattengale and Holloszy, 1967). Muller (1974), however, has found hypertrophy of the rat soleus, gastrocnemius and rectus femoris after 12 weeks using these same training regimens. Muller's findings do not differ very much from those studies previously reported. For example, as for most of the previously quoted studies, Muller used female rats and found no statistically significant difference for the body weights of endurance trained and control rats. Nevertheless, Muller believed he should use relative weights to evaluate hypertrophy. No significant hypertrophy, as judged by the absolute weight of the muscles, was reported. With trained male rats, which definitively differ in body weight when compared to control rats, absolute weights of the muscles cannot be used to assess hypertrophy unless the trained muscles were heavier than the non-trained muscles, which is usually not the case. Thus, relative weights were used in the present study, and significant hypertrophy of the muscles was observed. The only known study (Staudte et al., 1973) reporting the effect of sprint training on rat heart and muscles weights might have been too short (21 training days) to be conclusive. Nevertheless, no hypertrophy was found.

The use of organ to body weight ratios, as in the present study, has been questioned (Dowell et al., 1976;

Gollnick et al., 1967; Héroux and Gridgeman , 1958; Muller, 1974 and 1975a; Tanner, 1949). The heart weight, for example, is not linearly proportional to the body weight, and the use of heart to body weight ratios might reveal a false hypertrophy if, as is the case for male rats, there is a concomitant decrease in body weight. The use of female rats or food restriction techniques are often used to avoid this problem (Baldwin et al., 1977; Holloszy, 1967; Oscai et al., 1971). Otherwise, linear or better, logarithm regressed weights must be used (Dowell et al., 1976; Gollnick et al., 1967; Héroux and Gridgeman, 1958). However, the absence of significant correlation between the organ weights and the body weights made uncertain choices of a regressed weight equation. The lack of significant correlation was probably due to the small cell size. Thus, it was decided to use relative weight. As far as skeletal muscle is concerned, the use of muscle weight to body weight ratios is justified since muscle constitutes the major portion of the total body mass (Héroux and Gridgeman, 1958; Muller, 1974 and 1975).

There is one more assumption involved when relative weight or regressed weights are used to assess hypertrophy. It is assumed that the rest of the body, or more accurately, its comportments (fat and lean tissue), as compared to the studied organ, are changing in equal proportion. For example, if the fat is decreased as a results of training, which is probably the case in the present study,* the organ to body weight ratio will no longer indicate hypertrophy.

True absolute hypertrophy is not completely excluded and may be necessary to meet the overload since muscle cell proliferation is absent in adult muscle tissues. This seems to be the case in pathological myocardial hypertrophy where the contractile properties of the heart are depressed, as opposed to physical training which improves the contractile properties of the heart (Dowell et al., 1976; Hepp et al., 1974; Penpargkul and Scheuer, 1970) and often leads to myocardial hypertrophy as reported earlier. Skeletal muscle might incur some degree of hypertrophy with physical training as indicated by increased fiber area (Gollnick et al., 1973b; Gordon et al., 1967; Muller, 1974). This increase in cell size with chronic but intermittent exercise is probably within the optimal and critical cell size (Goss, 1966; Hubbard et al., 1974 and 1975) and differs from permanent compensatory overload hypertrophy (Baldwin, 1977b; Dowell et al., 1976). Of course, the relative proportion of sarcoplasmic and myofibrillar proteins may change without external hypertrophy with training (Gordon et al., 1967). Edington and Edgerton (1976, p. 230), Goldberg et al. (1975) and Muller (1974) have reported some occasional hyperplasia (fiber splitting or development of satelite cells) concomitant with skeletal muscle hypertrophy. In conclusion, both training programs used in this study appear to have increased the relative weight of the heart and skeletal muscle, but not of the liver.

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* Although fat % was not assessed in this study, the decrease in body weight after training is usually the result of a decrease in fat % (Booth, 1972; Crews et al., 1969; Mayer, 1968).

Lactate Dehydrogenase Adaptation to Chronic Exercise (Tables 12 to 17, Appendix F)

From the two way analysis of variance (Appendix F), all the organs of both training groups have lower LDH and M-LDH activities as compared to the sedentary group. As training might have an opposite effect on LDH in different organs (Baldwin _et_ _al_., 1972 and 1973; Gollnick _et_ _al_., 1961 and 1967; York _et_ _al_., 1974, 1975 and 1976) and as the analysis pools together all organs, a more stringent look at the results indicated that the main effect was due to changes in FT skeletal muscles and liver since either no change or a reversed trend was observed in the soleus and in the heart. One way analysis of variance conducted on each muscle revealed, however, only a few significant training effects (Tables 13 to 18). The fact that pooled data from the various muscles and liver resulted in significant effects in a greater number of cases, is acceptable from a statistical and mathematical point of view since pooling increases the degree of freedom and decreases the variance of the sample. Such a difference between one way and two way analyses of variance illustrates the necessity of larger sample groups, particularly when the coefficient of variation (CV) is large, which is the case with LDH activity ($CV = SD \times 100\overline{X}^{-1} = 30\%$).

Lactate Dehydrogenase Adaptation to Endurance Training. The present results are consistent with the literature which either reports similar effects or no change with the utilization of small sample groups (n ≤ 10). For instance, Baldwin et al. (1972 and 1973), Costill et al. (1976), Hickson et al. (1976), Karlsson et al. (1975), Suominen and Heikkinen (1975) and York et al. (1975) reported smaller LDH and M-LDH activity in FT muscles of endurance trained animals and man. In the heart, on the contrary, Gollnick et al. (1961 and 1967), Walpurger and Anger (1970), and York et al. (1975 and 1976) reported increased LDH and M-LDH activity. Other studies reported no significant effects of endurance training on the FT muscles (Bohmer, 1969; Bylund et al., 1977; Gollnick et al., 1967; Holloszy, 1971; Molé et al., 1973; Morgan et al., 1971) as well as the heart (Walpurger and Anger, 1970) and the ST soleus (Baldwin et al., 1973). It is interesting to note however that in all these cases, there was a tendency to follow the specific adaptation pattern reported previously. It seems that the small sample size used in these studies might have lead to a type II error. The lack of significant training effects might also be the result of different exercise conditions. For example, Walpurger and Anger (1970) reported a 15% and a 10% increase in myocardial LDH activity after endurance running and swimming respectively, but only the 15% increase was significant. Although the duration of the training regimen might affect the amplitude of the LDH changes as shown by York et al. (1975 and 1976), this does not seem to be the case for the present study where the training lasted two months longer than others reported in the literature even though the intensity (31m/min, 8% slope) was similar to other studies (Baldwin et al., 1973; Gollnick et al., 1970; Holloszy, 1979; York et al., 1974, 1975, 1976). However, the duration of the training session was at the lower range of the reviewed studies which might partly explain the few significant training effects observed (1X ANOVA). On the other hand, Gollnick et al. (1970) and Fitts et al. (1975) have reported improved oxidative capacity of rat skeletal muscles with running training sessions of similar intensity and duration. In any case, the general tendency with endurance training appears to be a LDH decrease in FT muscle, an increase in the heart, and an increase or no change in the ST soleus.

There might be some exceptions to this pattern of adaptation. Firstly, the fact that training did not change significantly the LDH activity in human muscles (Bylund et al., 1977; Morgan et al., 1971; Sjodin et al., 1976a and b) and the fact that endurance trained athletes have 30 to 68% less LDH activity than sedentary subjects, would suggest some kind of genetic selection. However, training effects are not excluded since athletes are usually in a trained state for many years whereas training studies usually last only a few weeks. Thus, duration of training regimen appears to be an important factor that has not been fully investigated. Again in each case reported above, there was a 6 to 22% decrease in human skeletal muscle LDH suggesting that the training regimen might have not been long enough to affect significant changes. This might also explain the apparent discrepancies between human and rat studies, where (in the latter case) endurance training significantly reduced the LDH activity of FT muscles as reported earlier. Indeed, the life span of a rat is much shorter than that of a human (1/15). A second fact that might also explain this human-rat controversy is that laboratory rats are relatively sedentary animals when restricted to normal cage activity whereas human control subjects are not. Thus the differences between "sedentary" controls and trained subjects might be greater and more easily significant in rats than in humans. In any case, all reviewed studies, whether dealing with human or other mammals, never show apposite trends . Only one study (Zika _et_ _al_., 1973) reported a significant increase in the biceps brachii of rats trained "tonically." The undescribed nature of the training regimen as well as the sampled muscle make further discussion uncertain.

At the beginning of the present study, it was believed that the use of a more specific LDH assay technique (see Methodology chapter and this chapter section entitled "Lactate Dehydrogenase in Various Tissues) would yield more conclusive results. However, as shown by the two way and one way analyses of variance, this study simply confirmed what has been previously reported for endurance training in FT muscles and the heart. It appears that the large coefficient of variation of the LDH values (30%) whether methodological or biological, as compared to other parameters (15% for PC and CPK), is a major problem in this study, and a larger "n" might improve the experimental design.

Heart muscle does not usually increase its oxidative capacity with endurance training (Baldwin et al., 1977a; Holloszy, 1975; Oscai et al., 1977b). The heart is continuously active and is probably closer to the genetic limits of its oxidative potential. It is therefore possible that any substantial increase in the work load and energy demand is met by an increased glycolytic capacity. In the skeletal muscles, the specific LDH adaptation may be explained by the specific recruitment of fiber types. With endurance running, glycogen is depleted faster in ST fibers as compared to FT fibers (Gollnick et al., 1973a and d). Also ST fibers are innervated by the smaller and more excitable motor neurons and are therefore the first to become active (Edington and Edgerton, 1976). Finally, the tension level might have been relatively higher in the ST fibers as compared to FT fibers or muscles. Therefore, the ST soleus has to increase or at least maintain both its oxidative and glycolytic capacities to meet the extra energy demand of endurance running. On the contrary, FT muscles working at a relatively lower intensity increase their oxidative capacity which results in a reduced LDH activity.

To summarize, it seems that endurance training increases Total LDH activity, and more specifically M-LDH of the heart, decreases activity in FT muscles and retains or possibly increases activity in the ST soleus.

Lactate Dehydrogenase Adaptation to Sprint Training. As stated earlier, both intermittent and continuous training groups showed similar LDH adaptations. High intensity, short duration (i.e. sprint) training is less well documented in the literature. Mixed skeletal muscles of sprint and strength trained athletes have been shown to have higher LDH and M-LDH (Costill et al., 1976; Karlsson et al., 1975). None of the sprint training studies on humans were able to demonstrate any significant LDH increases (Sjodin et al., 1976a and b; Thorstensson et al., 1975). On the other hand, sprint training in animals either kept LDH in mixed skeletal muscles at the same level (Staudte et al., 1973) or decreased it (Hickson et al., 1976a; York et al., 1974). These trends are similar to those reported in the present study. Sprint training has also been shown to induce opposite trends between humans (Costill et al., 1976; Thorstensson et al., 1975) and other mammals (Bagby et al., 1972; Saubert et al., 1973; Staudte et al., 1973) for other anaerobic enzymes, such as myosin ATPase, phosphorylase, triosephosphate dehydrogenase, PK, PFK, AK and CPK in mixed skeletal muscle. It must be said however that the equivalence of the sprint training regimens between animals and humans is not clear.

In humans, the energy sources of running at different speeds are well documented (Astrand and Rodahl, 1970, p. 314; Gollnick and Hermansen, 1973; Margaria, 1972). However, it is not certain that sprint training programs as used in animal studies (Bagby _et_ _al_., 1972; Fitts _et_ _al_., 1974; Hickson _et_ _al_., 1976a and b; Ruhling _et_ _al_., 1973; Saubert _et_ _al_., 1973; Staudte _et_ _al_., 1973) are mainly stressing the anaerobic metabolism as originally intended. The duration and the ratio of the work/rest intervals of these programs are quite different and the aerobic metabolism might have been the principal target in some of these training programs when the total duration of the work intervals were long enough. In the present study both forms of training, continuous at 31m/min and intermittent at 70-75m/min with a work/rest interval ratio of 1 min to 4 min, resulted in similar changes in body and organ weight. Also, it has been shown (Léger, 1975):1) that such intermittent training could be more easily done than the continuous form; 2) that peak blood lactate was similar in both forms of training in the rat whereas;3) humans can hardly double the speed at which they can run continuously for 1 hour, when training intermittently with the same work/rest intervals; 4) that humans had also higher blood lactate with this intermittent work as compared to the continuous form of running; and 5) that the blood lactate concentrations after such form of running (9 umole/ml) are somewhat lower than the ones reported by Baldwin _et_ _al_. (1977c) after a 5 min run at 48 m/min on a 17% slope (14.4 u mole/ml) and by Saubert et al. (1973) after 20 x 30 sec run at 67 m/min interspaced with 30 sec rest intervals (19.4 u mole/ml). This demonstrates that the intermittent training used in this study did not fully stress the anaerobic metabolism of these rats. More than the speed, the duration of the work/rest intervals appears to explain the lower blood lactate levels of the present study as the longer rest intervals (4 min) might have permitted the complete resynthesis of the PC stores before the start of the next work bout (Fox et al., 1969; Hultman et al., 1967a; Margaria, 1972; Piiper and Spiller, 1970). Fedak et al. (1974) have shown that the energy cost of running is double for bipeds as compared to quadripeds who are more efficient at higher speeds because they possess more gait options, passing from trot to gallop. The energy sources of running rats are unknown at the present time and may not follow the same pattern as humans. It has been reported that rats can run at 160 m/min (Guiness book of animal records), well above the speed used in this study. On the other hand, Ruhling et al. (1973) and Hickson et al. (1967a and b) have estimated the physiological limit at 100 m/min for rats in a running wheel. Saubert et al. (1973) have estimated 50 m/min to be the speed that corresponds to the $\dot{V}O_2$max of the rats. Nevertheless, Hickson et al. (1973) found similar decreases in LDH activity of FT and ST muscles with both sprint and endurance training with a concomitant increase in fumarase activity.

These authors used a work/rest interval ratio of 1/4 as in the present study with work intervals of 10 sec however, and the speed of the treadmill was set at 99 m/min. The intermittent training used by Staudte et al. (1973) was even closer to the present study: the speed was set at 80 m/min, slope, at 30°, work intervals, at 45 sec with at least 1 hour rest between the 4 repetitions. After 21 days of training, Staudte et al. (1973) observed no change in LDH of ST and FT muscles. In view of the half-life times for LDH (Fritz et al., 1969 and 1973), 21 days may have been too short to induce any LDH changes. Baldwin et al. (1977a) found greater oxidative capacity improvement when using continuous running with interspersed sprints as compared to steady state running training. Other studies disclosed no difference between sprint and endurance training in animals (Bagby et al., 1972; Fitts et al., 1974; Ruhling et al., 1973). This information suggests that some form of sprint training may stress the aerobic metabolism more than the anaerobic metabolism in rat muscles.

Saubert et al. (1973) did not study LDH, but reported an increase in other glycolytic enzyme activities (e.g. PFK, PH, PK) in the soleus but not in the red and white portions of the gastrocnemius. Staudte et al. (1973) also found increased glycolytic activity in the soleus but not in the fast rectus femoris. Even with endurance training, Baldwin et al. (1973) and Holloszy et al. (1975) found an increased glycolytic activity of the soleus instead of the usual decrease found in the mixed skeletal muscle. It seems that, with previously reported types of sprint training, the ST soleus behaves reciprocally to FT muscles for the same reasons discussed for endurance training. It is not excluded however that, with other forms (i.e. more strenuous) of sprint training, FT muscles also increase their glycolytic activity as suggested by the higher LDH activity of highly trained human sprinters (Costill _et_ _al_., 1976; Karlsson _et_ _al_., 1975; Sjodin _et_ _al_., 1976a; Thorstenson _et_ _al_., 1974).

Another point in relation to sprint training is the smaller total training time as opposed to continuous endurance training. This alone may explain the fewer significant LDH adaptations with sprint studies. LDH might be less rate limiting than other glycolytic enzymes and take more time to adapt. It has also been shown that LDH has a longer half-life than other soluble proteins (Don and Master, 1975; Fritz _et_ _al_., 1969; Schimke, 1973).

As far as liver is concerned, two way analyses of variance indicated that chronic exercise decreased its LDH activity. The decrease in total LDH was more closely related to M-LDH than the H-LDH. This is unexpected since liver is usually seen more as a site of lactate oxidation rather than a site of pyruvate reduction (Keul, 1973; Rowell, 1966 and 1971). However, 1 X ANOVA revealed no significant changes; therefore, one must be cautious in explaining any changes observed in liver LDH with training.

Metabolism of High Energy Compounds and Chronic Exercise.

Endurance and sprint training regimens used in this study failed to increase the high energy compound stores (ATP + PC) in any of the studied tissues (Tables 7 and 9), except for a slight ATP increase for the endurance group as seen from the pooled data from organs (Appendix F). On the other hand, intermittent training did reduce the ATP stores by 10 to 15% as compared to the other groups in all tissues except the heart (Table 7 and Appendix F). The training regimens were not more sufficient in increasing the CPK and AK activities (Table 10 and 11). On the contrary, CPK activities of the fast twitch muscles were generally depressed in both the continuous and intermittent training groups (Table 10 and Appendix F).

The concentration levels of high energy compounds and enzymes might not be very important limiting factors in the kind of work loads used in this study, assuming the overload principle of adaptation to training. The absence of changes in PC levels of the skeletal muscles after continuous and intermittent training is consistent with the findings of Karlsson _et_ _al_. (1972) and Thorstensson _et_ _al_. (1975) on endurance and sprint trained humans respectively. On the other hand, this lack of increase in PC stores as well as the slight decrease in ATP levels of the skeletal muscle of the sprint trained rats as opposed to the endurance trained rats are at variance with the findings of Russian workers (Yakolev, 1965; Yampolskaya, 1952 as quoted by Haralambie, 1972; and Rogozskin, 1976). Since the details of the experimental design of these authors are not known, it is hard to make any comment at the present time. FT and ST muscles appear to behave similarly in this study although reciprocal trends have previously been reported by Gale and Nagle (1971). The PC levels reported by these authors as well as their sampling technique and statistical design are however questionable. As far as ATP is concerned, Karlsson et al. (1972) indicated an increase after endurance training in human skeletal muscle, whereas Bohmer (1969) reported no changes in rat gastrocnemius after swimming training which is consistent with the present study. It is possible that these discrepancies simply reflect a statistical artifact (small "n" and large variance) since there was an increasing tendency in each case. The nature of the training regimen as well as the subjects (humans or animals) might also be involved. Nevertheless, it seems that endurance training either increases the ATP level of skeletal muscle or has no effect on it. With sprint training the present study revealed no training effect on muscle ATP.

In the heart, endurance exercise (Degenring et al., 1975 and Scheuer et al., 1970) and other forms of experimentally-induced hypertrophy (Rabinowitz and Zak, 1975) usually result in a decrease or no change in the ATP and PC levels. This is in accord with the present study which demonstrates no training effect on myocardial ATP and PC with either endurance or sprint running. Gangloff et al.

(1961) reported a PC increase in the heart with training but their sampling technique as well as the very low published values are questionable.

As far as CPK is concerned, the present study supports the absence of change found by Walpurger and Anger (1970) but is in opposition to the increase reported by Wagner and Critz (1970) in the heart of endurance trained animals. Heart CPK after sprint training does not seem to have been investigated elsewhere. In the ST soleus, the present findings support the absence of change reported by Dieter (1970) but again are opposed to the increase reported by Wagner and Critz (1970) after endurance training. Since details of Wagner and Critz's study (1970) were not explicited (i.e. abstract), it is worthless to speculate on their results. In sprint-trained rats, Staudte et al. (1973) found an increase in soleus CPK but not in the rectus femoris, whereas in the present study CPK was found to stay at the same level in the soleus and to decrease in FT muscles. This decrease of CPK in fast twitch muscles in sprint trained rats is also in opposition with the increase found in sprint-trained humans (Thorstensson et al., 1975). The 5 second duration of the sprint intervals used by Thorstensson et al. (1975) as opposed to 1 minute intervals in the present study might have imposed greater and more exclusive stress on the CPK reaction. This is supported by the concomitant absence of change in LDH and $\dot{V}O_2$max reported by the same authors. Also, as discussed earlier, sprint training regimens might not be equivalent in man and animals. The decrease found in FT skeletal muscles of endurance trained rats is also in opposition with the increase (Wagner and Critz, 1970) or the absence of change reported for endurance trained rats (Bohmer, 1969; Dieter, 1970; Oscai and Holloszy, 1971) and endurance trained humans (Suominen and Heikkinen, 1975).

To summarize, it seems that ST and FT muscles behave reciprocally with either an increase or no change in ST muscles and either a decrease or no change in FT muscles with training depending on the nature of the working loads.

The fact that AK did not show any change with continuous and intermittent training is consistent with findings of Oscai and Holloszy (1971) in the gastrocnemius of endurance trained rats and the findings of Dart and Holloszy (1969) in the heart of rats after experimental hypertrophy (arteriovenous fistula). On the other hand, Walpurger and Anger (1970) reported a 50% and 30% rise in cytoplasmic myocardial AK after endurance swimming and running training respectively. The present data indicate a nonsignificant 20% rise in heart AK after intermittent training. As was the case for LDH, the large coefficient of variation (30-50%) might have hidden a possible rise in heart AK. In this regard, it is interesting to note that Thorstensson et al. (1976a and b) reported either an increase or no change in strength trained human skeletal muscles on two different occasions using the same training regimen but different subjects. The only known sprint study carried out with humans (Thorstensson _et al._, 1975) is consistent with the absence of change in AK of FT skeletal muscles after intermittent training.

The absence of change in liver CPK and AK does not appear to be documented in the literature. However, this pattern seems consistent with the apparent lack of functional significance of liver CPK and AK in exercise.

The Effect of Chronic Exercise on Anaerobic Variables in Rat Tissues

Although the previous discussion revealed many unexplained discrepancies and many concurrences with literature data, there appears to be much more consistency when looking at the total metabolism. It seems that the usual increase in oxidative capacity after endurance training (Baldwin _et al._, 1972; Benzi _et al._, 1975; Fitts _et al._, 1975; Gollnick _et al._, 1970; Holloszy, 1967; Molé _et al._, 1973; Pattengale and Holloszy, 1967) is more important for fast twitch muscles and can explain the decrease in LDH, M-LDH% and CPK and the absence of change in AK and high energy compound stores. Such a reciprocal behavior between oxidative and non-oxidative enzymes has been previously reported by Pette _et al._ (1973). These authors found a decrease in LDH, M-LDH, CPK and AK activities concomitant to an increased oxidative activity in rabbit fast twitch muscles stimulated intermittently for 8 hours a day. On the other hand, the increase in the oxidative capacity might be less important in the heart (Baldwin et al., 1977a; Holloszy, 1975a; Oscai et al., 1971b) and in the soleus (Holloszy et al., 1975) which would explain the retention or the increase of LDH, M-LDH and CPK activities of these muscles. As Baldwin et al. (1972 and 1973) and Holloszy et al. (1975) found similar increases in the oxidative capacity of the soleus as compared to fast twitch muscles, a higher intensity of work for the soleus might also explain the LDH and CPK activity retention in this muscle.

That intermittent and continuous training have similar effects on LDH, CPK and AK activities and on PC stores suggests that many of the selected forms of "sprint training" in animal studies, might be closer to endurance or continuous training. It is not excluded however that more strenuous forms of sprint training would bring more specific effects.

CHAPTER VI

SUMMARY AND CONCLUSIONS

Summary

In view of the importance of anaerobic metabolism in some forms of physical activity, and in view of the few and conflicting related studies, it was decided to investigate the activities of the M and H forms of LDH, and the high energy compound stores (ATP + PC) and their regulatory enzymes (CPK and AK). These parameters were studied in the following tissues: liver, heart, slow twitch soleus, fast twitch gastrocnemius, plantaris and tibialis anterior of rats. Three experimental conditions were established: 1) a continuous endurance training program known to increase the oxidative capacity of the tissues; 2) a high speed intermittent training regimen using a 1 min work and a 4 min rest interval intended to stimulate both anaerobic glycolysis and high energy compound metabolism; and 3) a control or sedentary regimen restricting the rats to normal cage mobility.

The results indicated that similar adaptative changes occured for both training regimens. The forms of "sprint" training used with animals are still empirical and very unclear. Both training regimens resulted in a decreased activity of LDH, M-LDH, and CPK in the fast twitch muscles (tibialis anterior, plantaris, gastrocnemius), without altering their AK activity and PC stores. In the heart and slow twitch soleus, LDH, M-LDH, CPK and AK activities as well ad PC stores were retained after chronic exercise. No LDH, CPK and AK changes were present in the liver. Both training programs reduced body weight gain and increased the organ weight to body weight ratios of the muscles but not of the liver.

It seems that the increased oxidative capacity usually found with endurance training in rats (Baldwin et al., 1972; Benzi et al., 1975; Fitts et al., 1975; Gollnick et al., 1970; Holloszy, 1967; Molé et al., 1973; Pattengale and Holloszy, 1967) might explain the decrease in LDH, M-LDH% and CPK and the absence of change in AK and high energy compound stores. On the other hand, the absence of adaptation for the oxidative capacity of the heart as found by Baldwin et al. (1977a), Holloszy et al. (1975), Holloszy (1975) and Oscai et al. (1971b), would explain the retention or the increase of LDH, M-LDH and CPK activities. In the soleus, retention of LDH and CPK activities were explained by a higher relative intensity of work for this muscle as compared to other muscles since similar increases in the oxidative capacity of the slow twitch soleus and the fast twitch muscles were also reported after endurance training in rats (Baldwin et al., 1972 and 1973; Holloszy et al., 1975).

Conclusions

Within the limitations of this study, the following conclusions were drawn:

1. Non-oxidative metabolism in heart, liver and slow and fast twitch muscles is affected in a similar manner by high speed intermittent and low speed continuous training in the laboratory rat;

2. Slow twitch and fast twitch muscles appear to adjust their non-oxidative metabolism reciprocally with sprint and endurance training;

3. The large coefficient of variation found with some variables and different results found with the one way and two way (pooled data) analyses of variance indicate the need for larger sample size in future studies to avoid possible statistical artifact.

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APPENDIX A

LDH ELECTROPHORESIS

Appendix A contains an illustration of LDH isoenzyme separation with polyacrylamide gel electrophoresis (plate 2). From such a separation, M_4 and H_4 LDH were analysed at different pyruvate concentrations to find the respective optimal pyruvate concentration (Table 21 and Figure 6). For further details, see Chapter III, Methods and Procedures.

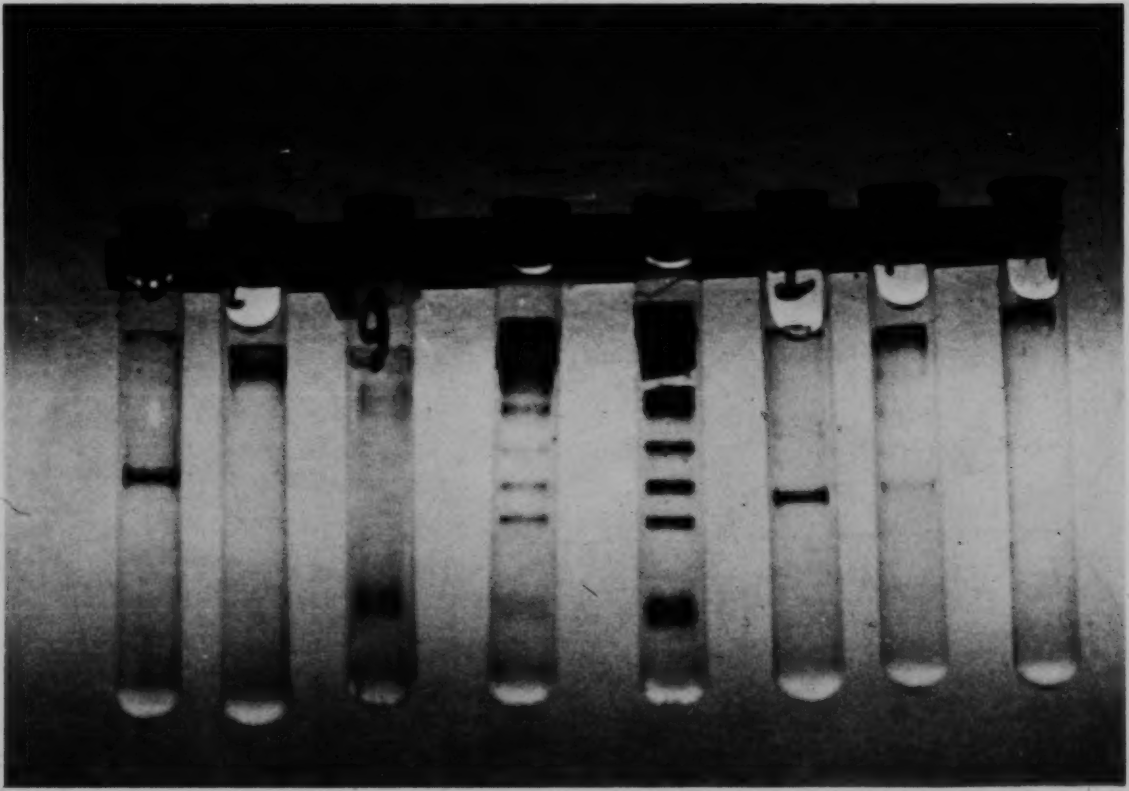

PLATE 2. LDH ISOENZYME SEPARATION WITH POLYACRYLAMIDE GEL ELECTROPHORESIS. From the 5 isoenzymes, H_4 is the fastest moving one toward the anode (bottom of the gels). From left to right: 1. LDH from beef heart (Sigma L 2625), 2. LDH from rabbit muscle (Sigma L 2500), 3, 4 and 5. Rat muscle homogenates, 6. H_4 (Sigma L3125), 7. Mixture of H_4 and M_4, 8. M_4 (Sigma L 2875).

TABLE 21 Optimal Pyruvate Concentrations for M_4 and H_4 LDH in the Rat.*

PA	H_4 LDH**		M_4 LDH**	
($X10^{-4}M$)	(ΔA/mn)	(%)	(ΔA/mn)	(%)
0	0	0	0	0
.5	0.065	29.5	0.010	25
1	0.130	69.2	0.015	37.5
3	0.220	100	0.031	77.5
5	0.217	98.4	0.036	90
10	0.220	100	0.040	100
20	0.182	82.5	0.040	100
40	0.111	50.5	0.029	72.7
100	0.065	29.6	0.020	50

* M_4 and H_4 were electrophoretically separated from heart and skeletal muscles of the rat.

** Average of three values.

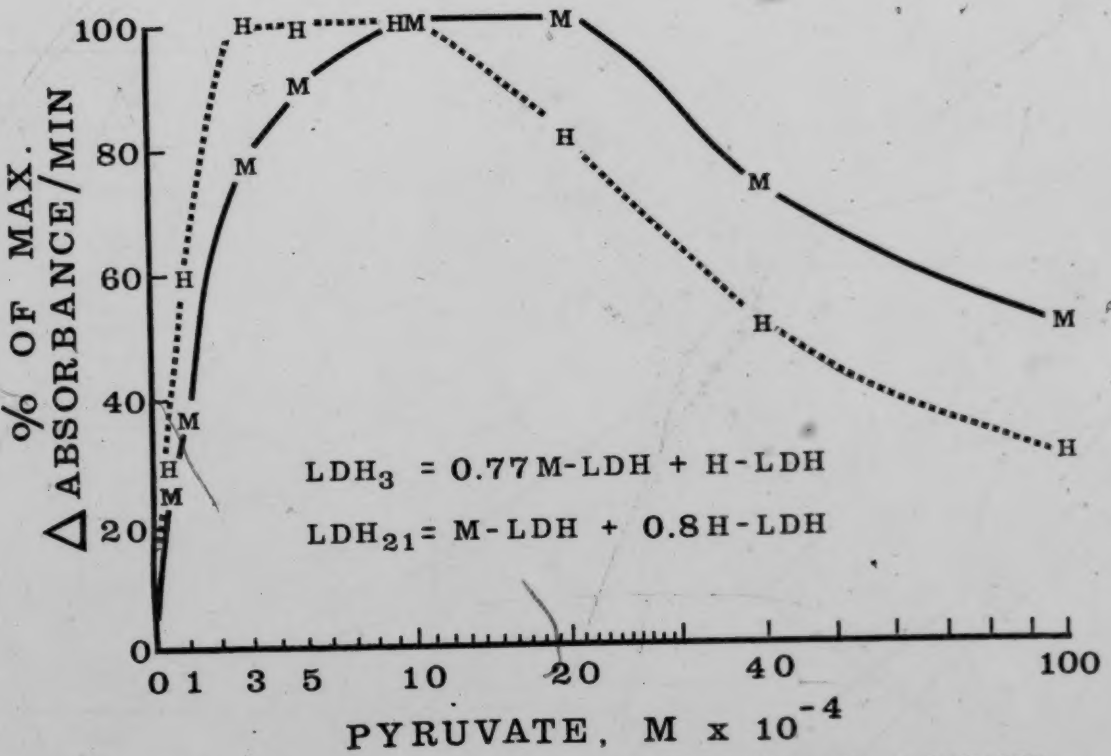

FIGURE 6. OPTIMAL PYRUVATE CONCENTRATIONS FOR M-LDH AND H-LDH IN RAT TISSUE

APPENDIX B

BODY WEIGHT PROGRESS FOR TRAINING GROUPS

TABLE 22 Body Weight Progress for Training Groups: Means and Standard Deviations.

AGE (week)	Sgr $\bar{X}$	Sgr SD	Cgr $\bar{X}$	Cgr SD	Igr $\bar{X}$	Igr SD
ARRIVAL[a]						
6	158.5	17.8	157.4	20.3	157.4	16.8
RUNNING EDUCATION						
7	204.5	13.9	203.4	17.4	205.7	14.6
8	255.6	19.2	258.4	19.2	256.8	16.5
9	290.7	20.1	293.3	18.2	293.5	19.5
10	305.0	26.5	317.5	18.0	311.7	20.4
TRAINING PERIOD						
11	341.8	25.4	343.0	20.2	330.5	37.4
12	358.8	26.5	362.0	20.7	361.7	26.9
13	390.0	28.6	383.0	26.9	375.1	27.8
14	410.5	32.5	384.5	37.8	386.5	25.6
15	433.9	31.7	395.5	36.9	399.4	26.8
16	459.3	38.0	420.5	35.2	419.6	26.6
17	476.0	36.1	430.4	41.6	426.5	26.2
18	489.2	40.4	438.5	40.6	432.2	28.0
19	504.9	40.0	457.5	36.4	439.8	25.5
20	507.7	48.4	459.0	29.2	453.3	30.0
21	527.1	45.1	475.1	29.5	460.2	32.2
22	535.9	41.4	478.6[b]	31.4[b]	465.2	35.0
23	553.7	50.9	487.2	34.3	471.9	33.1
24	558.8[b]	50.5[b]	495.6	33.4	478.2	35.7
25	568.9	50.9	503.0	35.7	473.4[b]	31.0[b]
26	572.6	52.4	513.6	37.1	481.9	28.0
27	571.7	54.4	516.0	37.3	488.5	36.7
33[d]	598.9	59.9	537.5[c]	37.7[c]	537.6[c]	54.2[c]
34	613.2	60.6	525.4	35.9	511.4	40.9
35	624.6[c]	69.7[c]	534.0	41.7	534.1	44.0

a. Groups: n = 10.
b. From that time and on, n = 9.
c. From that time and on, n = 8.
d. Subtitute trainer forgot to weigh rats.

APPENDIX C

RAW DATA

This appendix gives the raw data for each group, each organ and each variable in the following order:

1. Body weight
2. W_{abs}
3. W_{rel}
4. ATP
5. PC
6. ATP + PC
7. CPK
8. AK
9. LDH_{21}
10. LDH_3
11. LDH_{21}/LDH_3
12. M-LDH
13. H-LDH
14. TOTAL LDH

FINAL BODY WEIGHT (g)

Rat #	Igr	Cgr	Sgr
1	510	623	655
2	610	565	597
3	535	515	630
4	475	520	507
5	535	490	753
6	505	517	650
7	585	530	615
8	518	512	590

	W_{abs} (mg)		
	Igr	Cgr	Sgr
TA	870.600	1106.000	1006.600
	878.600	995.400	935.500
	1065.600	1023.600	789.800
	950.600	992.600	969.000
	1072.000	979.800	921.200
	1047.600	965.600	1194.200
	983.800	953.800	1080.000
	874.800	1010.200	1084.600
P	491.200	670.400	553.200
	464.600	620.000	652.400
	539.200	564.600	476.800
	518.600	566.400	583.800
	627.400	499.200	475.600
	616.400	623.400	626.600
	582.200	554.200	549.000
	565.200	556.400	575.200
GM	1209.400	1421.000	1335.800
	1280.000	1329.400	1180.700
	1177.600	1257.800	1079.000
	1040.400	1123.800	1172.800
	1357.800	1131.600	1026.000
	1298.200	1211.000	1327.200
	1238.200	1283.800	1186.000
	1170.200	1197.200	1244.000
GL	1696.000	1484.400	1508.200
	1477.800	1576.800	1313.300
	1564.400	1294.800	1174.200
	1178.200	1401.400	1607.400
	1607.400	1332.400	1234.000
	1402.600	1435.200	1576.000
	1492.400	1404.000	1590.400
	1182.000	1314.600	1514.200
S	0.000	294.600	282.800
	244.400	301.000	263.400
	201.000	244.000	192.800
	246.200	244.400	261.200
	239.900	225.600	167.800
	256.800	236.600	249.200
	304.800	253.200	317.600
	265.200	268.600	267.800
H	1602.800	1639.200	1496.000
	1416.000	1399.600	1460.500
	1558.000	0.000	1270.200
	1384.200	1707.000	0.000
	1497.000	1302.600	1745.100
	1500.000	1510.000	1469.000
	1533.800	1267.800	0.000
	0.000	0.000	1534.500
L	16472.300	15706.900	16564.300
	15615.000	15319.800	17400.000
	13950.000	11800.700	14773.900
	12025.300	14086.500	17823.400
	1278.340	11496.300	16968.200
	10940.000	14772.500	15188.000
	13583.600	14567.500	14100.700
	15246.700	13903.600	15153.900

		W_{rel} (mg/g)	
	Igr	Cgr	Sgr
TA	1.707	1.775	1.537
	1.440	1.762	1.567
	1.992	1.988	1.254
	2.001	1.909	1.911
	2.004	2.000	1.223
	2.074	1.868	1.837
	1.682	1.800	1.756
	1.689	1.973	1.838
P	.963	1.076	.845
	.762	1.097	1.093
	1.008	1.096	.757
	1.092	1.089	1.152
	1.173	1.019	.632
	1.221	1.206	.964
	.995	1.046	.893
	1.091	1.087	.975
GM	2.371	2.281	2.039
	2.098	2.353	1.978
	2.201	2.442	1.713
	2.190	2.161	2.313
	2.538	2.309	1.363
	2.571	2.342	2.042
	2.117	2.422	1.929
	2.259	2.338	2.109
GL	3.326	2.383	2.303
	2.423	2.791	2.200
	2.924	2.514	1.864
	2.480	2.695	3.170
	3.004	2.719	1.639
	2.777	2.776	2.425
	2.551	2.649	2.586
	2.282	2.568	2.566
S	0.000	.473	.432
	.401	.533	.441
	.376	.474	.306
	.518	.470	.515
	.448	.460	.223
	.508	.458	.383
	.521	.478	.516
	.512	.525	.454
H	3.143	2.631	2.284
	2.305	2.477	2.446
	2.912	0.000	2.016
	2.914	3.283	0.000
	2.798	2.658	2.318
	2.970	2.921	2.260
	2.622	2.392	0.000
	0.000	0.000	2.601
L	32.299	25.212	25.289
	25.598	27.115	29.146
	26.075	22.914	23.451
	25.316	27.089	35.155
	23.842	23.462	22.534
	21.663	28.574	23.366
	23.220	27.486	22.928
	29.433	27.155	25.685

ATP (mmoles/g)

	Igr	Cgr	Sgr
TA	4.770	6.610	6.000
	4.870	4.630	5.750
	4.700	6.250	5.430
	5.280	5.020	5.260
	5.420	7.300	6.690
	4.960	5.250	5.540
	4.210	5.280	6.200
	4.700	6.410	5.410
P	5.210	4.830	4.630
	4.360	4.830	4.860
	5.020	6.150	5.430
	4.440	4.710	4.620
	4.520	6.790	4.600
	4.120	0.000	4.710
	5.160	4.830	6.570
	3.850	4.990	5.420
GM	4.500	4.980	4.740
	4.570	5.150	4.950
	5.480	5.400	4.900
	4.890	6.460	4.340
	4.270	7.300	4.290
	3.400	5.790	5.850
	5.480	4.760	7.740
	3.260	6.560	4.840
GL	4.010	4.660	4.700
	4.140	4.950	4.580
	4.720	7.460	4.950
	4.240	5.340	4.790
	4.020	5.770	4.360
	4.570	4.530	4.360
	4.630	4.390	5.980
	3.870	4.990	4.910
S	3.430	3.770	3.120
	2.110	4.130	3.510
	3.350	4.080	3.540
	3.500	3.760	3.060
	3.410	4.930	3.560
	3.410	3.450	3.370
	2.700	3.720	3.150
	3.550	3.470	3.020
H	3.970	4.860	3.920
	4.170	4.520	3.550
	4.210	4.950	3.870
	4.020	4.790	0.000
	4.310	4.360	3.980
	3.960	3.510	3.640
	4.580	4.210	4.750
	5.020	0.000	4.100

PC (mmoles/g)

	Igr	Cgr	Sgr
TA	14.390	17.810	22.470
	19.330	18.700	25.430
	18.830	17.600	16.910
	0.000	22.790	18.890
	15.750	16.740	13.870
	0.000	19.670	14.600
	18.700	19.260	20.280
	14.150	0.000	0.000
P	11.950	14.530	12.670
	15.060	16.830	30.130
	19.390	14.910	14.990
	0.000	11.490	15.420
	14.560	13.880	11.830
	0.000	11.670	9.260
	18.530	9.180	17.070
	11.360	0.000	0.000
GM	17.420	12.780	15.650
	17.350	17.650	19.790
	19.730	13.600	15.440
	0.000	23.180	14.340
	14.370	17.700	11.450
	0.000	18.390	15.150
	20.390	14.330	16.500
	11.000	0.000	0.000
GL	10.080	14.670	16.680
	14.040	16.100	18.610
	18.820	14.850	13.060
	0.000	15.870	0.000
	11.770	16.380	15.160
	0.000	14.740	13.200
	13.490	8.440	11.740
	12.250	0.000	0.000
S	8.940	8.680	7.220
	6.610	12.720	11.940
	8.970	8.230	8.370
	0.000	5.960	8.550
	7.830	12.880	8.530
	0.000	13.230	10.540
	7.680	6.330	6.960
	0.000	0.000	0.000
H	2.770	6.460	3.730
	6.870	5.720	7.410
	4.370	4.970	4.560
	0.000	6.400	0.000
	6.020	5.440	4.290
	0.000	0.000	6.650
	3.750	6.650	4.910
	7.320	0.000	0.000

ATP & PC (mmoles/g)

	Igr	Cgr	Sgr
TA	19.160	24.420	28.470
	24.200	23.330	31.180
	23.530	23.850	22.340
	19.960	27.810	24.150
	22.910	24.040	20.560
	18.850	24.920	20.800
	0.000	24.540	25.690
	0.000	0.000	0.000
P	17.160	19.360	17.300
	19.420	21.660	34.990
	24.410	21.060	20.420
	19.080	16.200	20.040
	23.690	20.670	16.430
	15.210	14.530	15.830
	0.000	14.010	22.490
	0.000	0.000	0.000
GM	21.920	17.760	20.390
	21.920	22.800	24.740
	25.210	19.000	20.340
	18.640	29.640	18.680
	25.870	25.000	15.740
	14.260	24.180	22.890
	0.000	19.090	21.340
	0.000	0.000	0.000
GL	14.090	19.330	21.380
	18.180	21.050	23.190
	23.540	22.310	17.530
	15.790	21.210	0.000
	18.120	22.150	20.060
	16.120	19.270	19.180
	0.000	12.830	16.650
	0.000	0.000	0.000
S	12.370	12.450	10.340
	8.720	16.850	15.450
	12.320	12.310	11.910
	11.240	9.720	11.610
	10.380	17.820	12.090
	0.000	16.680	13.690
	0.000	10.050	9.980
	0.000	0.000	0.000
H	6.740	11.320	7.650
	11.040	10.240	10.960
	8.580	9.920	8.430
	10.330	11.200	0.000
	8.330	9.800	8.270
	12.340	6.870	11.400
	0.000	10.860	9.010
	0.000	0.000	0.000

AK (IU/g)

	Igr	Cgr	Sgr
TA	117.290	143.200	231.800
	133.400	206.840	0.000
	63.380	50.940	108.070
	141.710	44.760	79.990
	178.800	37.360	154.060
	138.940	174.020	0.000
	153.440	116.340	66.660
	0.000	201.930	155.250
P	115.040	131.400	204.000
	151.000	212.130	0.000
	49.520	56.370	113.910
	172.740	45.130	84.360
	138.900	40.810	121.230
	118.340	168.300	0.000
	132.450	120.610	49.660
	0.000	218.890	201.340
GM	113.070	138.200	179.800
	143.000	210.750	0.000
	40.570	47.800	97.830
	179.130	36.670	99.610
	143.800	36.740	123.820
	151.600	203.620	0.000
	109.410	125.560	43.990
	0.000	176.530	180.060
GL	114.640	101.200	189.600
	117.400	0.000	0.000
	38.690	46.430	95.230
	163.860	36.070	101.840
	134.650	32.990	112.180
	102.000	143.460	0.000
	117.600	114.560	45.810
	0.000	196.140	166.340
S	0.000	71.490	92.700
	70.630	0.000	0.000
	23.340	24.480	55.410
	112.110	24.910	65.060
	60.020	23.610	56.640
	58.980	83.800	0.000
	62.310	57.080	21.110
	0.000	75.510	80.030
H	69.240	74.430	83.100
	55.920	90.520	0.000
	34.550	30.600	58.530
	77.520	32.190	47.840
	63.940	32.350	69.040
	106.970	117.350	0.000
	90.460	57.880	33.900
	0.000	81.690	71.490
L	19.080	22.160	22.700
	15.080	0.000	0.000
	17.240	21.450	16.890
	17.730	20.140	15.250
	12.650	20.410	16.730
	29.770	21.920	0.000
	27.320	12.850	18.550
	0.000	13.730	24.810

CPK (IU/g)

	Igr	Cgr	Sgr
TA	2275.200	0.000	0.000
	2614.600	3226.500	0.000
	2066.200	2076.000	3288.400
	2910.200	2039.700	2793.500
	2020.300	2750.300	2486.200
	2170.500	2499.500	3000.900
	2741.600	2500.400	2344.200
	2569.500	2706.800	3295.200
P	2088.900	0.000	2890.600
	3018.500	2079.100	2412.500
	1829.900	3048.200	2781.900
	2111.300	1962.800	2228.300
	1628.000	2200.900	3085.300
	2165.900	2192.300	2706.700
	2377.900	2715.900	2885.900
	2638.100	2873.500	3265.800
GM	2147.700	0.000	2470.600
	2945.100	2246.200	3060.000
	2034.100	2551.900	2607.400
	2384.300	1797.400	2121.600
	2216.400	2105.900	3810.400
	2981.700	1916.700	3020.600
	1855.800	2947.200	3118.900
	2216.400	2804.800	3491.300
GL	1990.800	2020.200	2486.600
	1837.700	2020.200	2177.000
	1309.400	2001.500	2555.600
	2485.500	1728.800	2605.400
	1431.900	1846.300	2963.900
	2128.900	1374.800	3589.400
	2167.400	2087.500	2552.200
	1853.500	2608.700	3226.500
S	0.000	1108.000	1197.600
	1130.700	1196.500	549.200
	1028.900	1192.800	1105.400
	1114.400	954.900	987.700
	1039.500	1116.200	1228.900
	1174.100	816.100	1039.500
	1254.900	1247.200	1026.400
	1235.700	843.400	1167.000
H	855.200	1159.200	868.800
	776.300	767.900	669.900
	859.600	879.000	943.000
	817.800	689.800	702.400
	826.700	1014.500	1053.100
	1070.100	1061.900	636.500
	1073.000	692.900	958.800
	810.100	1039.500	660.000
L	9.020	15.360	12.490
	10.060	39.230	7.190
	7.250	14.820	9.110
	11.150	13.610	8.490
	13.640	6.870	14.420
	11.100	17.880	9.410
	14.550	14.590	10.030
	40.550	16.970	0.000

LDH_{21} (IU/g)

	Igr	Cgr	Sgr
TA	424.600	339.300	850.000
	654.600	525.700	636.500
	468.300	615.500	717.900
	636.000	415.100	642.200
	241.300	576.000	612.200
	517.700	248.700	358.900
	433.800	480.100	455.600
	375.600	413.900	423.700
P	499.200	447.200	702.900
	682.500	486.400	521.700
	553.400	752.200	0.000
	545.300	459.700	722.300
	472.700	617.400	635.400
	401.800	228.000	510.000
	454.100	451.700	668.300
	417.800	559.000	431.500
GM	0.000	424.700	489.900
	514.800	434.500	555.100
	488.000	541.200	0.000
	454.500	447.600	0.000
	457.100	470.000	625.800
	587.800	197.300	398.200
	280.600	439.500	488.200
	312.800	561.900	333.400
GL	496.200	277.600	494.900
	376.900	392.300	490.400
	368.000	415.100	611.600
	573.900	393.800	682.000
	0.000	384.400	515.800
	465.300	176.800	457.000
	385.900	366.700	447.600
	192.200	490.400	342.300
S	0.000	215.800	115.500
	109.900	0.000	196.100
	107.200	159.300	122.800
	167.800	115.900	178.000
	98.100	138.500	149.800
	107.200	61.300	100.000
	128.700	0.000	108.300
	97.100	0.000	76.500
H	325.600	545.300	209.000
	273.900	196.100	359.600
	273.900	310.900	254.000
	290.500	246.100	0.000
	211.800	343.800	383.500
	356.500	0.000	0.000
	284.400	195.100	240.400
	143.200	182.400	235.400
L	260.900	0.000	314.300
	403.900	0.000	260.900
	318.600	347.800	314.800
	268.400	261.800	297.700
	0.000	339.100	484.900
	0.000	148.200	353.100
	305.300	145.700	248.200
	244.200	214.800	0.000

LDH$_3$ (IU/g)

	Igr	Cgr	Sgr
TA	361.900	286.400	758.600
	573.000	473.700	555.000
	452.700	517.700	534.000
	498.700	355.400	494.100
	196.100	420.100	506.600
	399.700	176.900	325.600
	315.000	345.900	357.600
	300.100	332.500	358.900
P	457.000	329.500	615.000
	582.800	440.300	490.400
	509.100	628.000	0.000
	454.000	403.700	539.400
	375.600	531.200	594.100
	353.900	167.900	424.600
	343.300	379.200	511.500
	323.600	476.600	395.200
GM	0.000	374.700	468.400
	480.100	312.800	521.700
	461.500	480.000	0.000
	376.300	376.500	0.000
	432.800	369.000	482.800
	432.900	166.100	368.700
	229.700	313.900	394.800
	294.200	440.300	300.100
GL	454.100	254.000	454.500
	350.100	392.300	423.700
	337.900	377.700	538.400
	439.400	321.100	505.800
	0.000	328.400	465.200
	391.600	136.700	408.000
	293.900	282.000	355.800
	189.300	430.500	297.200
S	0.000	196.100	161.300
	104.500	0.000	196.100
	99.900	207.000	155.200
	185.900	131.100	158.900
	98.100	159.300	170.100
	129.100	60.400	100.000
	132.100	0.000	110.800
	94.100	0.000	90.200
H	450.100	608.000	211.700
	346.300	215.700	433.400
	370.900	349.600	339.900
	329.600	281.300	0.000
	260.900	402.700	440.800
	426.200	0.000	0.000
	321.200	201.300	263.100
	162.800	196.100	274.600
L	227.500	0.000	276.200
	387.900	0.000	227.500
	276.500	313.900	272.100
	221.900	232.900	214.800
	0.000	282.900	451.000
	0.000	119.200	309.900
	230.300	123.300	202.400
	202.000	169.700	0.000

LDH_{21}/LDH_3

	Igr	Cgr	Sgr
TA	1.170	1.180	1.120
	1.140	1.110	1.150
	1.030	1.190	1.340
	1.280	1.170	1.300
	1.230	1.370	1.210
	1.300	1.400	1.100
	1.380	1.390	1.270
	1.250	1.240	1.180
P	1.090	1.360	1.140
	1.170	1.100	1.060
	1.090	1.200	0.000
	1.200	1.140	1.340
	1.260	1.160	1.070
	1.140	1.360	1.200
	1.320	1.190	1.310
	1.290	1.170	1.090
GM	0.000	1.130	1.050
	1.070	1.390	1.060
	1.060	1.130	0.000
	1.210	1.190	0.000
	1.060	1.270	1.300
	1.360	1.190	1.080
	1.220	1.400	1.240
	1.060	1.280	1.110
GL	1.090	1.090	1.090
	1.080	1.000	1.160
	1.090	1.100	1.140
	1.310	1.230	1.340
	0.000	1.170	1.110
	1.190	1.310	1.120
	1.310	1.300	1.260
	1.020	1.140	1.150
S	0.000	1.100	.720
	1.050	0.000	1.000
	1.070	.770	.790
	.900	.880	1.120
	1.000	.870	.880
	.830	1.010	1.000
	.970	0.000	.980
	1.030	0.000	.870
H	.720	.900	.990
	.790	.910	.830
	.740	.890	.750
	.880	.870	0.000
	.810	.850	.870
	.840	0.000	0.000
	.890	.970	.910
	.880	.930	.860
L	1.150	0.000	1.140
	1.040	0.000	1.150
	1.150	1.110	1.160
	1.210	1.210	1.390
	0.000	1.200	1.080
	0.000	1.240	1.140
	1.330	1.180	1.230
	1.210	1.270	0.000

M - LDH (IU/g)

	Igr	Cgr	Sgr
TA	351.800	286.900	633.100
	510.900	382.100	501.300
	276.400	524.300	757.000
	617.300	340.600	643.000
	219.800	624.800	538.900
	515.500	279.100	256.300
	473.400	529.600	441.500
	352.900	385.200	355.700
P	347.900	478.100	549.200
	563.200	349.400	336.900
	380.500	650.500	0.000
	474.200	356.100	757.200
	448.500	501.100	417.000
	309.100	244.000	443.500
	467.300	386.300	674.700
	413.900	462.800	300.400
GM	0.000	325.400	299.900
	340.400	479.800	358.700
	309.400	409.400	0.000
	399.600	381.300	0.000
	288.700	455.200	623.900
	628.500	167.800	268.900
	252.200	490.600	448.900
	201.700	546.000	243.000
GL	346.100	193.800	341.900
	252.100	204.300	394.400
	254.400	294.100	471.000
	579.100	356.600	716.000
	0.000	316.900	374.100
	395.900	180.800	340.100
	392.700	367.400	424.400
	106.100	380.200	272.200
S	0.000	153.400	-35.300
	68.500	0.000	102.100
	71.000	-41.000	-3.500
	49.700	28.700	132.500
	51.100	28.800	35.700
	10.200	33.800	52.100
	59.900	0.000	51.200
	56.800	0.000	16.500
H	-89.800	153.400	103.200
	-8.200	61.300	33.500
	-59.400	81.300	-46.700
	69.800	54.800	0.000
	8.000	56.400	80.400
	40.500	0.000	0.000
	71.500	88.700	77.900
	33.700	66.500	40.900
L	205.500	0.000	243.100
	243.700	0.000	205.500
	253.600	251.800	252.900
	236.700	248.600	327.800
	0.000	293.700	323.200
	0.000	137.600	273.900
	315.300	122.600	224.700
	215.100	205.800	0.000

H - LDH (IU/g)

	Igr	Cgr	Sgr
TA	91.000	65.500	271.100
	179.600	179.500	169.000
	239.900	114.000	-48.900
	23.400	93.200	-1.000
	26.800	-61.000	91.700
	2.800	-38.000	128.300
	-49.500	-61.900	17.700
	28.400	35.900	85.000
P	189.100	-38.700	192.100
	149.200	171.300	231.000
	216.100	127.100	0.000
	88.900	129.500	-43.700
	30.300	145.300	273.000
	115.900	-19.900	83.100
	-16.600	81.700	-8.000
	4.930	120.200	163.900
GM	0.000	124.200	237.400
	218.000	-56.700	245.500
	223.300	164.800	0.000
	68.600	82.900	0.000
	210.500	18.500	2.400
	-51.300	36.900	161.700
	35.500	-63.800	49.200
	138.900	19.900	113.000
GL	187.600	104.800	191.200
	156.000	235.000	120.000
	142.000	151.200	175.700
	-6.500	46.500	-42.600
	0.000	84.400	177.200
	36.800	-2.500	146.100
	-8.400	-.900	29.000
	107.600	137.700	87.600
S	0.000	78.000	188.400
	51.800	0.000	117.500
	45.200	207.200	157.900
	147.600	109.000	56.900
	58.800	137.100	142.600
	121.200	34.400	59.900
	85.900	0.000	71.400
	50.300	0.000	77.500
H	519.200	439.900	132.200
	352.600	168.500	407.600
	416.700	287.000	375.800
	275.800	239.100	0.000
	254.700	359.300	378.900
	395.000	0.000	0.000
	266.200	133.000	203.100
	136.800	144.900	243.100
L	69.300	0.000	39.000
	200.300	0.000	69.300
	81.200	120.000	77.400
	39.700	41.400	-37.600
	0.000	56.800	202.200
	0.000	13.200	99.000
	-12.450	28.900	29.400
	36.400	11.200	0.000

TOTAL LDH (IU/g)

	Igr	Cgr	Sgr
TA	442.800	352.400	904.200
	669.500	561.600	670.300
	516.300	638.300	708.100
	640.700	433.700	642.000
	219.800	563.800	630.500
	518.300	241.100	384.500
	423.900	467.700	459.100
	381.300	421.100	440.700
P	537.000	439.500	741.300
	512.300	520.700	567.900
	596.600	777.600	0.000
	563.100	435.600	713.600
	478.800	646.400	690.000
	425.000	224.000	526.600
	450.800	468.000	666.700
	418.800	583.000	464.300
GM	0.000	449.500	537.400
	558.400	425.200	604.200
	532.700	574.200	0.000
	468.200	464.200	0.000
	499.200	473.700	626.300
	577.500	204.700	430.500
	287.700	426.700	498.000
	340.600	565.900	356.000
GL	533.700	298.600	533.100
	408.100	439.300	514.400
	396.400	445.300	646.700
	572.600	403.100	673.500
	0.000	401.300	551.200
	432.700	178.300	486.200
	384.200	366.500	453.400
	213.700	517.900	272.200
S	0.000	231.400	153.200
	120.300	0.000	219.600
	116.200	165.800	154.400
	197.300	137.700	139.400
	110.900	165.900	178.300
	131.400	68.200	112.000
	145.900	0.000	122.600
	107.200	0.000	94.000
H	429.400	643.300	235.400
	344.400	229.800	441.100
	357.200	368.300	329.200
	345.700	393.900	0.000
	262.700	415.700	459.300
	435.600	0.000	0.000
	337.600	221.700	281.000
	170.600	211.400	284.000
L	274.800	0.000	332.100
	444.000	0.000	274.800
	334.800	371.800	330.300
	276.300	290.100	290.200
	0.000	350.500	525.300
	0.000	150.800	372.900
	302.800	151.500	254.100
	251.500	217.000	0.000

APPENDIX D

TRAINING GROUP COMPARISONS FOR EACH DEPENDANT VARIABLE AND TISSUE: ONE WAY ANALYSES OF VARIANCE AND OTHER RELATED STATISTICS

This appendix contains only those among the 89 one way analyses of variance, that revealed significant differences between groups. Scheffe's contrasts are also shown. ANOVA Tables appears in the following order:

1. W_{abs} (BW)
2. W_{rel} (TA, P, GM, H)
3. ATP (TA, GM, GL, S)
4. CPK (P, GM, GL)
5. LDH_{21} (GL)
6. LDH_3 (GL)
7. M-LDH (H)

On the computer print-out, Sgr, Cgr and Igr are represented by No 3, 2, 1 respectively. "Moyenne" and "Ecart-type" are the mean and standard deviation. Scheffe's contrasts (alpha (j) - alpha (i)) are significant at $P < 0.05$ when their confidence interval are both positive or negative in which case group j is larger or smaller than group i respectively.

W_{ABS} - BW

```
N=  8 LES Y DU GROUPE SONT -
    510.0     611.0     545.0     475.0     535.0     505.0     585.0     518.0
N=  8 LES Y DU GROUPE SONT
    623.0     575.0     515.0     520.0     490.0     517.0     530.0     512.0
N=  8 LES Y DU GROUPE SONT
    655.0     597.0     630.0     507.0     753.0     650.0     615.0     590.0

GROUPE NUMERO  1       MOYENNE=   534.125     ECART-TYPE=    43.969
GROUPE NUMERO  2       MOYENNE=   534.000     ECART-TYPE=    41.006
GROUPE NUMERO  3       MOYENNE=   624.625     ECART-TYPE=    69.658
   MOYENNE GENERALE=      564.250

 ALPHA( 1)=    -30.125
 ALPHA( 2)=    -30.25
 ALPHA( 3)=     60.375

N=  8 LES RESIDUS DU GROUPE SONT
  -24.125    76.875    10.875   -59.125      .875   -29.125    50.875   -16.125
N=  8 LES RESIDUS DU GROUPE SONT
   89.000    41.000   -19.000   -14.000   -44.000   -17.000    -4.000   -22.000
N=  8 LES RESIDUS DU GROUPE SONT
   30.375   -27.625     5.375  -117.625   128.375    25.375    -9.625   -34.625
                                      SOURCE        SS          DL            S            F
                                   *************************************************************
                                      ENTRE LES    .43742E+05      2        .21871E+05     7.698
                                       GROUPES
                                      ERREUR       .59603E+05     21        .28411E+04
                                   *************************************************************
                                      TOTAL        .10340E+06     23

LA VALEUR CALCULEE DE PHI EST...    2.265

CALCUL DES CONTRASTES DE SCHEFFE CAR L'HYPOTHESE H0 EST REJETEE AVEC COMME FFF    3.470

  ALPH( 2) - ALPH( 1) = ( -70.3334,  70.0834)

  ALPH( 3) - ALPH( 1) = (  20.2912, 160.7188)
  ALPH( 3) - ALPH( 2) = (  20.4162, 160.8338)
```

W_{rel} - TA

```
I=   3
LES N(I)=   8   8   8

N=  8 LES Y DU GROUPE SONT
      1,7        1,4        2,0        2,0        2,0        2,1        1,7        1,7
N=  8 LES Y DU GROUPE SONT
      1,8        1,8        2,0        1,9        2,0        1,9        1,8        2,0
N=  8 LES Y DU GROUPE SONT
      1,5        1,6        1,3        1,9        1,2        1,8        1,8        1,8

GROUPE NUMERO  1      MOYENNE=       1,824      ECART-TYPE=        ,225
GROUPE NUMERO  2      MOYENNE=       1,884      ECART-TYPE=        ,098
GROUPE NUMERO  3      MOYENNE=       1,615      ECART-TYPE=        ,267
   MOYENNE GENERALE=        1,774

 ALPHA( 1)=         ,049
 ALPHA( 2)=         ,110
 ALPHA( 3)=        -,159

N=  8 LES RESIDUS DU GROUPE SONT
     -,117      -,384       ,168       ,177       ,180       ,250      -,142      -,135
N=  8 LES RESIDUS DU GROUPE SONT
     -,109      -,122       ,10[illegible]       ,025       ,116      -,016      -,084       ,089
N=  8 LES RESIDUS DU GROUPE SONT
     -,078      -,048      -,361       ,296      -,392       ,222       ,141       ,223

                                    SOURCE        SS           DL          MS           F
                                 ***********************************************************

                                   ENTRE LES    ,31845E+00      2       ,15923E+00     3,635
                                    GROUPES

                                    ERREUR      ,91976E+00     21       ,43798E-01
                                 ***********************************************************

                                    TOTAL       ,12382E+01     23

LA VALEUR CALCULEE DE PHI EST      1,557

CALCUL DES CONTRASTES DE SCHEFFE CAR L'HYPOTHESE HO EST REJETEE AVEC COMME FFF      3,4668

  ALPH( 2) - ALPH( 1) = (   -,2148,    ,3363)

  ALPH( 3) - ALPH( 1) = (   -,4838,    ,0673)
  ALPH( 3) - ALPH( 2) = (   -,5445,    ,0065)
```

$V_{rel} \geq V_p$

```
 I=    3
 LES N(I)=    8    8    8

N=  8 LES Y DU GROUPE SONT
     1.0         .8        1.0        1.1        1.2        1.2        1.0        1.1
N=  8 LES Y DU GROUPE SONT
     1.1        1.1        1.1        1.1        1.0        1.2        1.0        1.1
N=  8 LES Y DU GROUPE SONT
      .8        1.1         .8        1.2         .6        1.0         .9        1.0

GROUPE NUMERO  1      MOYENNE=      1.038     ECART-TYPE=        .142
GROUPE NUMERO  2      MOYENNE=      1.090     ECART-TYPE=        .054
GROUPE NUMERO  3      MOYENNE=       .914     ECART-TYPE=        .171
   MOYENNE GENERALE=        1.014

 ALPHA( 1)=        .024
 ALPHA( 2)=        .076
 ALPHA( 3)=       -.100

N=  8 LES RESIDUS DU GROU E SONT
    -,075      -,276      -,030       ,054       ,135       ,183      -,043       ,053
N=  8 LES RESIDUS DU GROUPE SONT
    -,013       ,008       ,007      -,000      -,070       ,117      -,043      -,002
N=  8 LES RESIDUS DU GROUPE SONT
    -,059       ,179      -,157       ,238      -,282       ,050      -,021       ,061
                                          SOURCE        SS          DL          MS          F
                                         **************************************************************

                                          ENTRE LES   ,13046E+00      2        ,65229E-01      3,733
                                          GROUPES

                                          ERREUR      ,30699E+00     21        ,17476E-01
                                         **************************************************************

                                          TOTAL       ,49745E+00     23

LA VALEUR CALCULEE DE PHI EST       1,577

CALCUL DES CONTRASTES DE SCHEFFE CAR L"HYPOTHESE HO EST REJETEE AVEC COMME FFF      3,4668

  ALPH( 2) - ALPH( 1) = (   -,1227,    ,2254)

  ALPH( 3) - ALPH( 1) = (   -,2983,    ,0498)
  ALPH( 3) - ALPH( 2) = (   -,3097,   -,0016)
```

W_{rel} - GM

```
 T=   3
LES N(I)=   8   8   8
N=   8 LES Y DU GROUPE SONT
      2,4        2,1        2,2        2,2        2,5        2,6        2,1        2,3
N=   8 LES Y DU GROUPE SONT
      2,3        2,4        2,4        2,2        2,3        2,3        2,8        2,3
N=   8 LES Y DU GROUPE SONT
      2,0        2,0        1,7        2,3        1,4        2,0        1,9        2,1

GROUPE NUMERO  1     MOYENNE=      2,293     ECART-TYPE=       ,182
GROUPE NUMERO  2     MOYENNE=      2,331     ECART-TYPE=       ,087
GROUPE NUMERO  3     MOYENNE=      1,936     ECART-TYPE=       ,286
  MOYENNE GENERALE=       2,187

 ALPHA( 1)=        ,107
 ALPHA( 2)=        ,144
 ALPHA( 3)=       -,251

N=   8 LES RESIDUS DU GROUPE SONT
     ,078       -,195      -,092      -,103       ,245       ,278      -,176      -,034
N=   8 LES RESIDUS DU GROUPE SONT
    -,050        ,022       ,111      -,170      -,022       ,011       ,091       ,007
N=   8 LES RESIDUS DU GROUPE SONT
     ,103        ,042      -,223       ,377      -,573       ,106      -,007       ,173
                                       SOURCE        SS            DL         MS            F
                                  ************************************************************
                                       ENTRE LES   ,76100E+00      2       ,38050E+00      9,296
                                        GROUPES
                                       ERREUR      ,85954E+00      21      ,40930E-01
                                  ************************************************************
                                       TOTAL       ,16205E+01      23

LA VALEUR CALCULEE DE PHI EST      2,489

CALCUL DES CONTRASTES DE SCHEFFE CAR L"HYPOTHESE HO EST REJETEE AVEC COMME FFF     3,4668

  ALPH( 2) - ALPH( 1) = (    -,2285,     ,3042)

  ALPH( 3) - ALPH( 1) = (    -,6237,    -,0910)
  ALPH( 3) - ALPH( 2) = (    -,6616,    -,1289)
```

W_{rel} - H

```
 I=     3
 LES N(I)=    7    6    6
N=  7 LES Y DU GROUPE SONT
      3.1        2.3        2.9        2.9        2.8        3.0        2.6
N=  6 LES Y DU GROUPE SONT
      2.6        2.5        3.3        2.7        2.9        2.4
N=  6 LES Y DU GROUPE SONT
      2.3        2.4        2.0        2.3        2.3        2.6

GROUPE NUMERO  1      MOYENNE=       2,809      ECART-TYPE=       ,273
GROUPE NUMERO  2      MOYENNE=       2,727      ECART-TYPE=       ,327
GROUPE NUMERO  3      MOYENNE=       2,321      ECART-TYPE=       ,196
   MOYENNE GENERALE=        2,629

 ALPHA( 1)=        ,180
 ALPHA( 2)=        ,098
 ALPHA( 3)=       -,308

N=  7 LES RESIDUS DU GROUPE SONT
     ,334       -,504       ,103       ,105       -,011       ,161       -,187
N=  6 LES RESIDUS DU GROUPE SONT
    -,096       -,250       ,556      -,069        ,194      -,335
N=  6 LES RESIDUS DU GROUPE SONT
    -,037        ,125      -,305      -,003       -,061       ,280
                                      SOURCE        SS            DL          MS           F
                                    ***************************************************************

                                      ENTRE LES    ,85458E+00      2         ,42729E+00     5,814
                                       GROUPES

                                      ERREUR       ,11758E+01      16        ,73490E-01
                                    ***************************************************************

                                      TOTAL        ,20304E+01      18

LA VALEUR CALCULEE DE PHI EST       1,969


CALCUL DES CONTRASTES DE SCHEFFE CAR L"HYPOTHESE HO EST REJETEE AVEC COMME FFF     3,6337


  ALPH( 2) - ALPH( 1) = (     -,4887,      ,3204)
                                         3.
  ALPH( 3) - ALPH( 1) = (     -,8949,     -,0817)
  ALPH( 3) - ALPH( 2) = (     -,8281,      ,0158)
```

ATP - TA

```
 I=    3
 LES N(I)=   8   8   8
N=  8 LES Y DU GROUPE SONT
      4,8       4,9       4,7       5,3       5,4       5,0       4,2       4,7
N=  8 LES Y DU GROUPE SONT
      6,6       4,6       6,3       5,0       7,3       5,3       5,3       6,4
N=  8 LES Y DU GROUPE SONT
      6,9       5,8       5,4       5,3       6,7       5,5       6,2       5,4

GROUPE NUMERO  1      MOYENNE=      4,864     ECART-TYPE=       ,375
GROUPE NUMERO  2      MOYENNE=      5,844     ECART-TYPE=       ,927
GROUPE NUMERO  3      MOYENNE=      5,785     ECART-TYPE=       ,484
   MOYENNE GENERALE=        5,497

 ALPHA( 1)=        -,634
 ALPHA( 2)=         ,346
 ALPHA( 3)=         ,288

N=  8 LES RESIDUS DU GROUPE SONT
     -,094      ,006     -,164      ,416      ,556      ,096     -,654     -,164
N=  8 LES RESIDUS DU GROUPE SONT
      ,766    -1,214      ,406     -,824     1,456     -,594     -,564      ,566
N=  8 LES RESIDUS DU GROUPE SONT
      ,215     -,035     -,355     -,525      ,905     -,245      ,415     -,375
                                   SOURCE         SS          DL         MS          F
                                *************************************************************

                                 ENTRE LES    ,48335E+01       2       ,24167E+01    5,875
                                  GROUPES

                                  ERREUR      ,86384E+01      21       ,41135E+00
                                *************************************************************

                                  TOTAL       ,13472E+02      23

LA VALEUR CALCULEE DE PHI EST       1,979

CALCUL DES CONTRASTES DE SCHEFFE CAR L"HYPOTHESE HO EST REJETEE AVEC COMME FFF      3,4668


 ALPH( 2) - ALPH( 1) = (     ,1356,     1,8244)

  ALPH( 3) - ALPH( 1) = (     ,0768,     1,7657)
  ALPH( 3) - ALPH( 2) = (    -,9032,      ,7857)
```

ATP = GM

```
I=      3
LES N(I)=    8     8     8
N=  8 LES Y DU GROUPE SONT 5.5       4,9       4,3       3,4       5,5       3,3
      4,5        4,6
N=  8 LES Y DU GROUPE SONT 5,4       6,5       7,3       5,8       4,8       6,6
      5,0        5,2
N=  8 LES Y DU GROUPE SONT 4,9       4,3       4,3       5,9       7,7       4,8
      4,7        5,0

GROUPE NUMERO  1      MOYENNE=     4.481    ECART-TYPE=      .835
GROUPE NUMERO  2      MOYENNE=     5.400    ECART-TYPE=      .890
GROUPE NUMERO  3      MOYENNE=     5,206    ECART-TYPE=     1,130
  MOYENNE GENERALE=        5,163

 ALPHA( 1)=        -.681
 ALPHA( 2)=         .638
 ALPHA( 3)=         .044

N=  8 LES RESIDUS DU GROUPE SONT     .409      -.211    -1,081       .399    -1,221
      .019       .089       .909
N=  8 LES RESIDUS DU GROUPE SONT     .660      1,500     -.010     -1,040       .760
     -.820      -.650      -.400
N=  8 LES RESIDUS DU GROUPE SONT    -.866      -.916      .644      2,534      -.366
     -.466      -.256      -.306
                                SOURCE        SS          DL          MS           F
                            ****************************************************************

                                ENTRE LES    .69794E+01      2       .34897E+01      3,775
                                 GROUPES

                                ERREUR       .19410E+02     21       .92431E+00
                            ****************************************************************

                                TOTAL        .26390E+02     23

LA VALEUR CALCULEE DE PHI EST      1,586

CALCUL DES CONTRASTES DE SCHEFFE CAR L'HYPOTHESE HO EST REJETEE AVEC COMME FFF     3,4668

 ALPH( 2) - ALPH( 1) = (     .0530,    2,5845)

 ALPH( 3) - ALPH( 1) = (   -.5408,    1,9908)
 ALPH( 3) - ALPH( 2) = (  -1,8595,     .6720)
```

ATP - OL

```
T=   3
LES N(I)=    8    8    8

N=  8 LES Y DU GROUPE SONT
     4,0        4,1        4,7         4,2         4,0         4,6         4,6        3,9
N=  8 LES Y DU GROUPE SONT
     4,7        5,0        7,5         5,3         5,8         4,5         4,4        5,0
N=  8 LES Y DU GROUPE SONT
     4,7        4,6        5,0         4,8         4,8         4,4         6,0        4,9

GROUPE NUMERO  1      MOYENNE=      4,275     ECART-TYPE=       ,323
GROUPE NUMERO  2      MOYENNE=      5,261     ECART-TYPE=       ,995
GROUPE NUMERO  3      MOYENNE=      4,829     ECART-TYPE=       ,516
   MOYENNE GENERALE=         4,788

 ALPHA( 1)=       -,513
 ALPHA( 2)=        ,473
 ALPHA( 3)=        ,040

N=  8 LES RESIDUS DU GROUPE SONT
    -,265      -,135       ,445       -,035       -,255       ,295       ,355      -,405
N=  8 LES RESIDUS DU GROUPE SONT
    -,601      -,311      2,199        ,079        ,509      -,731      -,871      -,271
N=  8 LES RESIDUS DU GROUPE SONT
    -,129      -,249       ,121       -,039       -,469      -,469      1,151       ,081
                                        SOURCE       SS           DL          MS            F
                                  ************************************************************

                                        ENTRE LES   ,39104E+01      2        ,19552E+01      4,312
                                         GROUPES

                                        ERREUR      ,95212E+01     21        ,45339E+00
                                  ************************************************************

                                        TOTAL       ,13432E+02     23

LA VALEUR CALCULEE DE PHI EST      1,696

CALCUL DES CONTRASTES DE SCHEFFE CAR L"HYPOTHESE HO EST REJETEE AVEC COMME FFF      3,4668

  ALPH( 2) - ALPH( 1) = (     ,0997,    1,8728)

  ALPH( 3) - ALPH( 1) = (    -,3328,    1,4403)
  ALPH( 3) - ALPH( 2) = (  -1,3190,     ,4540)
```

ATP - S

```
I=    3
LES N(I)=    8    8    8

N=  8 LES Y DU GROUPE SONT
     3.4       2.1       3.4        3.5        3.4        3.4        2.7        3.6
N=  8 LES Y DU GROUPE SONT
     3.8       4.1       4.1        3.8        4.9        3.5        3.7        3.5
N=  8 LES Y DU GROUPE SONT
     3.1       3.5       3.5        3.1        3.6        3.4        3.2        3.0

GROUPE NUMERO  1     MOYENNE=      3.183     ECART-TYPE=      .509
GROUPE NUMERO  2     MOYENNE=      3.914     ECART-TYPE=      .478
GROUPE NUMERO  3     MOYENNE=      3.291     ECART-TYPE=      .228
   MOYENNE GENERALE=       3.463

 ALPHA( 1)=      -.280
 ALPHA( 2)=       .451
 ALPHA( 3)=      -.171

N=  8 LES RESIDUS DU GROUPE SONT
     .248     -1.073      .168       .318       .228       .228      -.482       .368
N=  8 LES RESIDUS DU GROUPE SONT
    -.144       .216      .166      -.154      1.016      -.464      -.194      -.444
N=  8 LES RESIDUS DU GROUPE SONT
    -.171       .219      .249      -.231       .269       .079      -.141      -.271
                                    SOURCE          SS             DL            MS              F
                                  ***************************************************************

                                    ENTRE LES    .24908E+01        2         .12454E+01       6.920
                                     GROUPES

                                    ERREUR       .37772E+01       21         .17987E+00
                                  ***************************************************************

                                    TOTAL        .62681E+01       23

LA VALEUR CALCULEE DE PHI EST      2.148

CALCUL DES CONTRASTES DE SCHEFFE CAR L'HYPOTHESE HO EST REJETEE AVEC COMME FFF      3.4668

  ALPH( 2) - ALPH( 1) = (     .1729,    1.2896)

  ALPH( 3) - ALPH( 1) = (    -.4496,     .6671)
  ALPH( 3) - ALPH( 2) = (   -1.1809,    -.0641)
```

```
                                   CPK  -  P

 T=   3
 LES N(I)=   8   7   8

N=  8 LES Y DU GROUPE SONT
   2088,9    3018,5    1829,9    2111,3    1628,0    2165,9    2377,9    2638,1

N=  7 LES Y DU GROUPE SONT
   2079,1    3048,2    1962,8    2200,9    2192,3    2715,9    2873,5

N=  8 LES Y DU GROUPE SONT
   2890,6    2412,5    2781,9    2228,3    3085,3    2706,7    2885,9    3265,8


GROUPE NUMERO  1      MOYENNE=  2232,313      ECART-TYPE=   442,509
GROUPE NUMERO  2      MOYENNE=  2438,957      ECART-TYPE=   430,201
GROUPE NUMERO  3      MOYENNE=  2782,125      ECART-TYPE=   337,517
   MOYENNE GENERALE=    2486,443


 ALPHA( 1)=    -254,131
 ALPHA( 2)=     -47,486
 ALPHA( 3)=     295,682


N=  8 LES RESIDUS DU GROUPE SONT
 -143,413    786,188  -402,412  -121,012  -604,312   -66,412   145,588   405,788
N=  7 LES RESIDUS DU GROUPE SONT
 -359,857    609,243  -476,157  -238,057  -246,657   276,943   434,543
N=  8 LES RESIDUS DU GROUPE SONT
  108,475   -369,625     -,225  -553,825   303,175   -75,425   103,775   483,675

                          SOURCE        SS             DL          MS           F
                       ****************************************************************

                          ENTRE LES   ,12319E+07       2        ,61593E+06      3,757
                           GROUPES

                          ERREUR      ,32786E+07      20        ,16393E+06
                       ****************************************************************

                          TOTAL       ,45104E+07      22


LA VALEUR CALCULEE DE PHI EST      1,583

CALCUL DES CONTRASTES DE SCHEFFE CAR L'HYPOTHESE HO EST REJETEE AVEC COMME FFF      3,4928


  ALPH( 2) - ALPH( 1) = (-347,1924, 760,4817)

  ALPH( 3) - ALPH( 1) = (  14,7551,1084,8649)
  ALPH( 3) - ALPH( 2) = (-210,6692, 897,0049)
```

```
                                        CPK  -  GM

 I=    3
 LES N(I)=    8    7    8

N=  8 LES Y DU GROUPE SONT
   2147.7     2945.1     2034.1     2384.3     2216.4     2981.7     1855.8     2216.4
N=  7 LES Y DU GROUPE SONT
   2246.2     2551.9     1797.4     2105.9     1916.7     2947.2     2804.8
N=  8 LES Y DU GROUPE SONT
   2470.6     3060.0     2607.4     2121.6     3810.4     3020.6     3118.9     3491.3

GROUPE NUMERO  1      MOYENNE=  2347.688      ECART-TYPE=   409.699
GROUPE NUMERO  2      MOYENNE=  2338.586      ECART-TYPE=   440.987
GROUPE NUMERO  3      MOYENNE=  2962.600      ECART-TYPE=   509.166
   MOYENNE GENERALE=    2558.800

 ALPHA( 1)=    -211.112
 ALPHA( 2)=    -220.214
 ALPHA( 3)=     403.800

N=  8 LES RESIDUS DU GROUPE SONT
 -199.987    597.413   -313.587    36.613   -131.287    634.013   -491.887   -131.287
N=  7 LES RESIDUS DU GROUPE SONT
  -92.386    213.314   -541.186   -232.686   -421.886    608.614    466.214
N=  8 LES RESIDUS DU GROUPE SONT
 -492.000     97.400   -355.200   -841.000    847.800     58.000    156.300    528.700
                                       SOURCE         SS           DL           MS           F
                                   ****************************************************************
                                       ENTRE LES    .20004E+07      2         .10002E+07     4.492
                                        GROUPES
                                       ERREUR       .44529E+07     20         .22264E+06
                                   ****************************************************************
                                       TOTAL        .64533E+07     22

LA VALEUR CALCULEE DE PHI EST      1.731

CALCUL DES CONTRASTES DE SCHEFFE CAR L"HYPOTHESE HO EST REJETEE AVEC COMME FFF      3.4928


  ALPH( 2) - ALPH( 1) = (-654.5483, 636.3448)

 ALPH( 3) - ALPH( 1) = (  -8.6481,1238.4731)
 ALPH( 3) - ALPH( 2) = ( -21.4323,1269.4608)
```

```
                                   CPK  -  GL

 I=     3
 LES N(I)=    8    8    8
N=   8 LES Y DU GROUPE SONT
   1990,8     1837,7     1309,4     2485,5     1431,9     2128,9     2167,4     1853,5
N=   8 LES Y DU GROUPE SONT
   2020,2     2020,2     2001,5     1728,8     1846,3     1374,8     2087,5     2608,7
N=   8 LES Y DU GROUPE SONT
   2486,6     2177,0     2555,6     2605,4     2963,9     3589,4     2552,2     3226,5

GROUPE NUMERO  1       MOYENNE=  1900,638     ECART-TYPE=   387,099
GROUPE NUMERO  2       MOYENNE=  1961,000     ECART-TYPE=   349,463
GROUPE NUMERO  3       MOYENNE=  2769,575     ECART-TYPE=   458,237
   MOYENNE GENERALE=   2210,404

 ALPHA( 1)=     -309,767
 ALPHA( 2)=     -249,404
 ALPHA( 3)=      559,171

N=   8 LES RESIDUS DU GROUPE SONT
   90,163   -62,937  -591,237   584,863  -468,737   228,263   266,763   -47,137
N=   8 LES RESIDUS DU GROUPE SONT
   59,200    59,200    40,500  -232,200  -114,700  -586,200   126,500   647,700
N=   8 LES RESIDUS DU GROUPE SONT
 -282,975  -592,575  -213,975  -164,175   194,325   819,825  -217,375   456,925
                                   SOURCE       SS            DL           MS            F
                               ***********************************************************

                                   ENTRE LES    ,37666E+07     2          ,18833E+07     11,723
                                    GROUPES

                                   ERREUR       ,33737E+07     21         ,16065E+06
                               ***********************************************************

                                   TOTAL        ,71403E+07     23

LA VALEUR CALCULEE DE PHI EST        2,796

CALCUL DES CONTRASTES DE SCHEFFE CAR L'HYPOTHESE HO EST REJETEE AVEC COMME FFF       3,4668

  ALPH( 2) - ALPH( 1) = (-467,3412, 588,0662)

  ALPH( 3) - ALPH( 1) = ( 341,2338,1396,6412)
  ALPH( 3) - ALPH( 2) = ( 280,8713,1336,2787)
```

```
                                        LDH   -  g1
                                           21

 T=   3
 LES N(I)=    7    8    8

N=  7 LES Y DU GROUPE SONT
   496,2     376,9     368,0      573,9      465,3      385,9      192,2

N=  8 LES Y DU GROUPE SONT
   277,6     392,3     415,1      393,8      384,4      178,8      366,7      490,4

N=  8 LES Y DU GROUPE SONT
   494,9     490,4     611,6      682,0      515,8      457,0      447,6      342,3


GROUPE NUMERO  1      MOYENNE=   408,343      ECART-TYPE=   121,250
GROUPE NUMERO  2      MOYENNE=   362,388      ECART-TYPE=    94,449
GROUPE NUMERO  3      MOYENNE=   505,200      ECART-TYPE=   103,644
   MOYENNE GENERALE=     426,048


 ALPHA( 1)=     -17,705
 ALPHA( 2)=     -63,660
 ALPHA( 3)=      79,152


N=  7 LES RESIDUS DU GROUPE SONT
   87,857    -31,443    -40,343   165,557     56,957    -22,443   -216,143
N=  8 LES RESIDUS DU GROUPE SONT
  -84,787     29,913     52,713    31,413     22,013   -183,588      4,313    128,013
N=  8 LES RESIDUS DU GROUPE SONT
  -10,300    -14,800    106,400   176,800     10,600    -48,200    -57,600   -162,900

                                     SOURCE        SS            DL          MS            F
                                 *******************************************************************

                                     ENTRE LES    ,84736E+05      2        ,42368E+05     3,752
                                      GROUPES

                                     ERREUR       ,22585E+06     20        ,11292E+05
                                 *******************************************************************

                                     TOTAL        ,31058E+06     22


LA VALEUR CALCULEE DE PHI EST       1,582


CALCUL DES CONTRASTES DE SCHEFFE CAR L"HYPOTHESE HO EST REJETEE AVEC COMME FFF      3,4928


  ALPH( 2) - ALPH( 1) = (-191,3164,  99,4057)


  ALPH( 3) - ALPH( 1) = ( -48,5039, 242,2182)
  ALPH( 3) - ALPH( 2) = (   2,3604, 283,2446)
```

LDH$_3$ - GL

```
T=   3
LES N(I)=   7   8   8

N=  7 LES Y DU GROUPE SONT
   454,1     350,1     337,9      439,4      391,6      293,9      189,3
N=  8 LES Y DU GROUPE SONT
   254,0     392,3     377,7      321,1      328,4      136,7      282,0      430,5
N=  8 LES Y DU GROUPE SONT
   454,5     423,7     538,4      505,8      465,2      408,0      355,8      297,2

GROUPE NUMERO  1     MOYENNE=   350,900      ECART-TYPE=     90,987
GROUPE NUMERO  2     MOYENNE=   315,338      ECART-TYPE=     92,631
GROUPE NUMERO  3     MOYENNE=   431,075      ECART-TYPE=     78,369
   MOYENNE GENERALE=     366,417

 ALPHA( 1)=    -15,517
 ALPHA( 2)=    -51,080
 ALPHA( 3)=     64,658

N=  7 LES RESIDUS DU GROUPE SONT
  103,200     -,800   -13,000     88,500     40,700   -57,000  -161,600
N=  8 LES RESIDUS DU GROUPE SONT
  -61,337    76,963    62,363      5,763     13,063  -178,638   -33,337   115,163
N=  8 LES RESIDUS DU GROUPE SONT
   23,425    -7,375   107,325     74,725     34,125   -23,075   -75,275  -133,875
                                         SOURCE        SS            DL          MS           F
                                      ***************************************************************

                                         ENTRE LES    ,56004E+05     2         ,28002E+05     3,667
                                          GROUPES

                                         ERREUR       ,15273E+06    20         ,76364E+04
                                      ***************************************************************

                                         TOTAL        ,20873E+06    22

LA VALEUR CALCULEE DE PHI EST       1,564

CALCUL DES CONTRASTES DE SCHEFFE CAR L'HYPOTHESE HO EST REJETEE AVEC COMME FFF      3,4928

  ALPH( 2) - ALPH( 1) = (-155,0984,   83,9734)

  ALPH( 3) - ALPH( 1) = ( -39,3609, 199,7109)
  ALPH( 3) - ALPH( 2) = (    ,2548, 231,2202)
```

13.

```
                                          M - IDH - H

 I=   3
LES N(I)=   8   7   6
N=  8 LES Y DU GROUPE SONT
   -89,8      -8,2      -59,4       69,8       8,0       40,5       71,5       33,7
N=  7 LES Y DU GROUPE SONT
   153,4      61,3       81,3       54,8      56,4       88,7       66,5
N=  6 LES Y DU GROUPE SONT
   103,2      33,5      -46,7       80,4      77,9       40,9

GROUPE NUMERO  1     MOYENNE=      8,263     ECART-TYPE=    58,478
GROUPE NUMERO  2     MOYENNE=     80,343     ECART-TYPE=    34,612
GROUPE NUMERO  3     MOYENNE=     48,200     ECART-TYPE=    53,328
   MOYENNE GENERALE=      43,700

 ALPHA( 1)=     -35,438
 ALPHA( 2)=      36,643
 ALPHA( 3)=       4,500

N=  8 LES RESIDUS DU GROUPE SONT
  -98,063    -16,463    -67,663    61,538      -,263     32,238     63,238     25,438
N=  7 LES RESIDUS DU GROUPE SONT
   73,057    -19,043       ,957   -25,543    -23,943      8,357    -13,843
N=  6 LES RESIDUS DU GROUPE SONT
   55,000    -14,700    -94,900    32,200     29,700     -7,300
                                     SOURCE          SS            DL            MS            F
                                   ********************************************************************

                                     ENTRE LES     ,19567E+05      2          ,97835E+04      3,884
                                      GROUPES

                                     ERREUR        ,45345E+05     18          ,25192E+04
                                   ********************************************************************

                                     TOTAL         ,64912E+05     20

LA VALEUR CALCULEE DE PHI EST       1,609

CALCUL DES CONTRASTES DE SCHEFFE CAR L'HYPOTHESE HO EST REJETEE AVEC COMME FFF      3,5546

  ALPH( 2) - ALPH( 1) = (   2,8194, 141,3413)

  ALPH( 3) - ALPH( 1) = ( -32,3362, 112,2112)
  ALPH( 3) - ALPH( 2) = (-106,5961,  42,3104)
```

APPENDIX E

ORGAN COMPARISONS FOR EACH DEPENDANT VARIABLE ON COMBINED DATA FROM ALL TRAINING GROUPS: ONE WAY ANALYSES OF VARIANCE AND OTHER RELATED STATISTICS

This appendix contains organ comparisons: one way analysis of variance and Scheffé's contrasts. The computer print-outs appear in the following order:

1. W_{abs}
2. W_{rel}
3. ATP
4. PC
5. ATP + PC
6. CPK
7. AK
8. LDH_{21}
9. LDH_3
10. LDH_{21}/LDH_3
11. M-LDH
12. H-LDH
13. TOTAL LDH

Legend for the computer print outs:

Group No.	Organ
1	TA
2	P
3	GM
4	GL
5	S
6	H
7	L

Moyenne = Mean
Ecart-type = Standard deviation

For the Scheffé's contrasts (alpha (j) - alpha (i)), two positive (or negative) limits of the confidence interval ($P < 0.05$) indicates that organ j is bigger (or smaller) than organ i.

W_{abs}

```
GROUPE NUMERO  1     MOYENNE=   909.646    ECART-TYPE=    83.850
GROUPE NUMERO  2     MOYENNE=   564.667    ECART-TYPE=    57.218
GROUPE NUMERO  3     MOYENNE=  1219.934    ECART-TYPE=    99.813
GROUPE NUMERO  4     MOYENNE=  1431.734    ECART-TYPE=   151.571
GROUPE NUMERO  5     MOYENNE=   253.430    ECART-TYPE=    35.448
GROUPE NUMERO  6     MOYENNE=  1409.121    ECART-TYPE=   132.329
GROUPE NUMERO  7     MOYENNE= 14114.060    ECART-TYPE=  3276.097
  MOYENNE GENERALE=   2924.717


   SOURCE        SS          DL  .      MS          F
*******************************************************************

  ENTRE LES   .35549E+10      5      .59248E+09   359.986
   GROUPES

   ERREUR     .24821E+09    155      .16014E+07
*******************************************************************

   TOTAL      .38031E+10    151
```

```
ALPH( 2) - ALPH( 1) = ( -1739.3213,   889.3530)

ALPH( 3) - ALPH( 1) = ( -1084.0338,  1544.6505)
ALPH( 3) - ALPH( 2) = (  -659.0547,  1969.6297)

ALPH( 4) - ALPH( 1) = (  -872.2338,  1756.4505)
ALPH( 4) - ALPH( 2) = (  -447.2547,  2181.4297)
ALPH( 4) - ALPH( 3) = ( -1102.5422,  1526.1422)

ALPH( 5) - ALPH( 1) = ( -2064.7671,   592.3363)
ALPH( 5) - ALPH( 2) = ( -1639.7879,  1017.3155)
ALPH( 5) - ALPH( 3) = ( -2295.0754,   362.0200)
ALPH( 5) - ALPH( 4) = ( -2506.8754,   150.2200)

ALPH( 6) - ALPH( 1) = (  -898.6635,  1097.6159)
ALPH( 6) - ALPH( 2) = (  -473.6063,  2322.5951)
ALPH( 6) - ALPH( 3) = ( -1128.9738,  1667.3076)
ALPH( 6) - ALPH( 4) = ( -1340.7738,  1455.5076)
ALPH( 6) - ALPH( 5) = (  -175.8162,  2647.1075)

ALPH( 7) - ALPH( 1) = ( 11810.0720, 14438.7563)
ALPH( 7) - ALPH( 2) = ( 12235.0512, 14363.7355)
ALPH( 7) - ALPH( 3) = ( 11579.7637, 14208.4430)
ALPH( 7) - ALPH( 4) = ( 11367.8627, 13996.6430)
ALPH( 7) - ALPH( 5) = ( 12532.0779, 15189.1313)
ALPH( 7) - ALPH( 6) = ( 11225.7903, 14023.0795)
```

W_{rel}

```
GROUPE NUMERO  1     MOYENNE=     1.774     ECART-TYPE=      .232
GROUPE NUMERO  2     MOYENNE=     1.014     ECART-TYPE=      .147
GROUPE NUMERO  3     MOYENNE=     2.187     ECART-TYPE=      .265
GROUPE NUMERO  4     MOYENNE=     2.557     ECART-TYPE=      .371
GROUPE NUMERO  5     MOYENNE=      .453     ECART-TYPE=      .075
GROUPE NUMERO  6     MOYENNE=     2.629     ECART-TYPE=      .335
GROUPE NUMERO  7     MOYENNE=    26.000     ECART-TYPE=     3.242
   MOYENNE GENERALE=      5.342
```

```
  SOURCE        SS           DL         MS           F
************************************************************

  ENTRE LES   .12111E+03      6      .20184E+04  1249.647
   GROUPES

  ERREUR      .25035E+03    155      .16152E+01
************************************************************

  TOTAL       .12361E+05    161
```

```
ALPH( 2) - ALPH( 1) = (    -2.0806,      .5594)

ALPH( 3) - ALPH( 1) = (     -.9078,     1.7322)
ALPH( 3) - ALPH( 2) = (     -.1472,     2.4928)

ALPH( 4) - ALPH( 1) = (     -.5272,     2.1128)
ALPH( 4) - ALPH( 2) = (      .2334,     2.8735)
ALPH( 4) - ALPH( 3) = (     -.9393,     1.7007)

ALPH( 5) - ALPH( 1) = (    -2.6555,      .0131)
ALPH( 5) - ALPH( 2) = (    -1.8949,      .7737)
ALPH( 5) - ALPH( 3) = (    -3.0676,     -.3991)
ALPH( 5) - ALPH( 4) = (    -3.4493,     -.7797)

ALPH( 6) - ALPH( 1) = (     -.5496,     2.2587)
ALPH( 6) - ALPH( 2) = (      .2110,     3.0193)
ALPH( 6) - ALPH( 3) = (     -.9618,     1.8465)
ALPH( 6) - ALPH( 4) = (    -1.3425,     1.4659)
ALPH( 6) - ALPH( 5) = (      .7581,     3.5933)

ALPH( 7) - ALPH( 1) = (    22.9058,    25.5453)
ALPH( 7) - ALPH( 2) = (    23.6664,    26.3065)
ALPH( 7) - ALPH( 3) = (    22.4937,    25.1237)
ALPH( 7) - ALPH( 4) = (    22.1130,    24.7530)
ALPH( 7) - ALPH( 5) = (    24.2127,    25.0813)
ALPH( 7) - ALPH( 6) = (    21.9671,    24.7755)
```

ATP

```
GROUPE NUMERO  1     MOYENNE=      5.497      ECART-TYPE=      .765
GROUPE NUMERO  2     MOYENNE=      4.985      ECART-TYPE=      .713
GROUPE NUMERO  3     MOYENNE=      5.162      ECART-TYPE=     1.071
GROUPE NUMERO  4     MOYENNE=      4.788      ECART-TYPE=      .764
GROUPE NUMERO  5     MOYENNE=      3.453      ECART-TYPE=      .522
GROUPE NUMERO  6     MOYENNE=      4.239      ECART-TYPE=      .445
   MOYENNE GENERALE=       4.693
```

```
   SOURCE        SS            DL          MS            F
**************************************************************

  ENTRE LES   .63831E+02       5       .12775E+02    23.022
   GROUPES

   ERREUR     .74920E+02      135      .55496E+00
**************************************************************

   TOTAL      .13880E+03      140
```

```
ALPH( 2) - ALPH( 1) = (    -1.2469,      .2214)


ALPH( 3) - ALPH( 1) = (    -1.0613,      .3913)
ALPH( 3) - ALPH( 2) = (     -.5564,      .9119)


ALPH( 4) - ALPH( 1) = (    -1.4355,      .0171)
ALPH( 4) - ALPH( 2) = (     -.9306,      .5377)
ALPH( 4) - ALPH( 3) = (    -1.1005,      .3521)


ALPH( 5) - ALPH( 1) = (    -2.7613,    -1.3087)
ALPH( 5) - ALPH( 2) = (    -2.2564,     -.7031)
ALPH( 5) - ALPH( 3) = (    -2.4263,     -.9737)
ALPH( 5) - ALPH( 4) = (    -2.0521,     -.5995)


ALPH( 6) - ALPH( 1) = (    -2.0015,     -.5162)
ALPH( 6) - ALPH( 2) = (    -1.4965,      .0042)
ALPH( 6) - ALPH( 3) = (    -1.6665,     -.1812)
ALPH( 5) - ALPH( 4) = (    -1.2923,      .1929)
ALPH( 6) - ALPH( 5) = (      .0335,     1.5188)
```

PC

```
                                                                    ALPH( 2) - ALPH( 1) = (    -6.8150,      -.3202)


                                                                    ALPH( 3) - ALPH( 1) = (    -5.2408,      1.2448)
                                                                    ALPH( 3) - ALPH( 2) = (    -1.6678,      4.3178)


                                                                    ALPH( 4) - ALPH( 1) = (    -7.3858,      -.8154)
                                                                    ALPH( 4) - ALPH( 2) = (    -3.2128,      2.7576)
                                                                    ALPH( 4) - ALPH( 3) = (    -5.2378,      1.1825)


GROUPE NUMERO  1     MOYENNE=     18.309     ECART-TYPE=      3.031  ALPH( 5) - ALPH( 1) = (   -12.6374,     -6.0570)
GROUPE NUMERO  2     MOYENNE=     14.736     ECART-TYPE=      4.571  ALPH( 5) - ALPH( 2) = (    -9.0544,     -2.4940)
GROUPE NUMERO  3     MOYENNE=     16.311     ECART-TYPE=      3.110  ALPH( 5) - ALPH( 3) = (   -10.6394,     -4.0690)
GROUPE NUMERO  4     MOYENNE=     14.208     ECART-TYPE=      2.574  ALPH( 5) - ALPH( 4) = (    -8.5786,     -1.9245)
GROUPE NUMERO  5     MOYENNE=      8.956     ECART-TYPE=      2.233
GROUPE NUMERO  6     MOYENNE=      5.451     ECART-TYPE=      1.359
   MOYENNE GENERALE=      13.151
                                                                    ALPH( 6) - ALPH( 1) = (   -16.1796,     -9.5163)
                                                                    ALPH( 6) - ALPH( 2) = (   -12.6066,     -5.9433)
                                                                    ALPH( 6) - ALPH( 3) = (   -14.1016,     -7.5103)
                                                                    ALPH( 6) - ALPH( 4) = (   -12.1203,     -5.3744)
  SOURCE        SS          DL          MS           F              ALPH( 6) - ALPH( 5) = (    -6.0637,      -.1228)
**************************************************************

  ENTRE LES   .22020E+04     5      .44039E+03    48.096
   GROUPES

  ERREUR      .10072E+04    110     .91565E+01
**************************************************************

  TOTAL       .32092E+04    115
```

ATP + PC

```
                                                                    ALPH( 2) - ALPH( 1) = (    -7.5557,     -.5193)

                                                                    ALPH( 3) - ALPH( 1) = (    -5.7832,     1.2532)
                                                                    ALPH( 3) - ALPH( 2) = (    -1.7457,     5.2907)

                                                                    ALPH( 4) - ALPH( 1) = (    -8.2481,    -1.1197)
                                                                    ALPH( 4) - ALPH( 2) = (    -4.2106,     2.9173)
                                                                    ALPH( 4) - ALPH( 3) = (    -5.9831,     1.1453)

GROUPE NUMERO  1     MOYENNE=     23.735     ECART-TYPE=      3.094    ALPH( 5) - ALPH( 1) = (   -14.8797,    -7.7513)
GROUPE NUMERO  2     MOYENNE=     19.693     ECART-TYPE=      4.634    ALPH( 5) - ALPH( 2) = (   -10.8422,    -3.7130)
GROUPE NUMERO  3     MOYENNE=     21.471     ECART-TYPE=      3.723    ALPH( 5) - ALPH( 3) = (   -12.6147,    -5.4853)
GROUPE NUMERO  4     MOYENNE=     19.052     ECART-TYPE=      3.028    ALPH( 5) - ALPH( 4) = (   -10.2412,    -3.0220)
GROUPE NUMERO  5     MOYENNE=     12.420     ECART-TYPE=      2.595
GROUPE NUMERO  6     MOYENNE=      9.647     ECART-TYPE=      1.640
  MOYENNE GENERALE=       17.772
                                                                    ALPH( 6) - ALPH( 1) = (   -17.6529,   -10.5245)
                                                                    ALPH( 6) - ALPH( 2) = (   -13.6154,    -6.4370)
                                                                    ALPH( 6) - ALPH( 3) = (   -15.3079,    -8.2595)
  SOURCE         SS            DL          MS           F           ALPH( 6) - ALPH( 4) = (   -13.0143,    -5.7951)
*************************************************************       ALPH( 6) - ALPH( 5) = (    -6.3828,      .8355)

  ENTRE LES   .28837E+04       5       .57774E+03    53.585
   GROUPES

   ERREUR     .11950E+04     111       .10782E+02
*************************************************************

   TOTAL      .40855E+04     116
```

CPK

```
GROUPE NUMERO  1      MOYENNE=  2589.319      ECART-TYPE=    405.879
GROUPE NUMERO  2      MOYENNE=  2406.443      ECART-TYPE=    432.791
GROUPE NUMERO  3      MOYENNE=  2553.000      ECART-TYPE=    541.602
GROUPE NUMERO  4      MOYENNE=  2210.404      ECART-TYPE=    557.173
GROUPE NUMERO  5      MOYENNE=  1075.739      ECART-TYPE=    165.012
GROUPE NUMERO  6      MOYENNE=   870.250      ECART-TYPE=    155.543
GROUPE NUMERO  7      MOYENNE=    14.252      ECART-TYPE=      3.608
   MOYENNE GENERALE=   1673.570


   SOURCE        SS            DL          MS            F
*************************************************************

  ENTRE LES   .14475E+09       6        .24125E+08   164.666
  GROUPES

   ERREUR     .22563E+08      154       .14651E+06
*************************************************************

   TOTAL      .16732E+09      160
```

```
ALPH( 2) - ALPH( 1) = (  -518.5810,   312.0290)

ALPH( 3) - ALPH( 1) = (  -446.2244,   385.1864)
ALPH( 3) - ALPH( 2) = (  -333.7912,   478.5042)

ALPH( 4) - ALPH( 1) = (  -790.4661,    32.6363)
ALPH( 4) - ALPH( 2) = (  -677.9340,   125.0554)
ALPH( 4) - ALPH( 3) = (  -750.2905,    53.4989)

ALPH( 5) - ALPH( 1) = ( -1928.2853, -1096.8745)
ALPH( 5) - ALPH( 2) = ( -1815.8520, -1003.5557)
ALPH( 5) - ALPH( 3) = ( -1808.2085, -1075.9132)
ALPH( 5) - ALPH( 4) = ( -1535.5597,  -731.7703)

ALPH( 6) - ALPH( 1) = ( -2130.6203, -1307.5178)
ALPH( 6) - ALPH( 2) = ( -2018.0802, -1214.2938)
ALPH( 6) - ALPH( 3) = ( -2090.4447, -1235.6553)
ALPH( 5) - ALPH( 4) = ( -1737.7504,  -942.5579)
ALPH( 6) - ALPH( 5) = (  -608.3838,   195.4056)

ALPH( 7) - ALPH( 1) = ( -2990.7727, -2159.3619)
ALPH( 7) - ALPH( 2) = ( -2878.3394, -2066.0441)
ALPH( 7) - ALPH( 3) = ( -2950.6959, -2138.4006)
ALPH( 7) - ALPH( 4) = ( -2598.0471, -1794.2577)
ALPH( 7) - ALPH( 5) = ( -1468.5351,  -656.3397)
ALPH( 7) - ALPH( 6) = ( -1257.8930,  -454.1036)
```

```
AK

GROUPE NUMERO  1     MOYENNE=   123.405     ECART-TYPE=    55.570
GROUPE NUMERO  2     MOYENNE=   126.005     ECART-TYPE=    57.042
GROUPE NUMERO  3     MOYENNE=   122.931     ECART-TYPE=    56.403
GROUPE NUMERO  4     MOYENNE=   103.535     ECART-TYPE=    49.501
GROUPE NUMERO  5     MOYENNE=    58.906     ECART-TYPE=    25.779
GROUPE NUMERO  6     MOYENNE=    65.691     ECART-TYPE=    24.910
GROUPE NUMERO  7     MOYENNE=    19.473     ECART-TYPE=     4.544
   MOYENNE GENERALE=      90.802


    SOURCE         SS           DL          MS          F
 *************************************************************

    ENTRE LES   .21814E+05      6       .36357E+05    18.874
     GROUPES

    ERREUR      .26198E+05     136      .19253E+04
 *************************************************************

    TOTAL       .48012E+05     142
```

```
ALPH( 2) - ALPH( 1) = (   -51.3061,    46.3489)

ALPH( 3) - ALPH( 1) = (   -54.3803,    43.2742)
ALPH( 3) - ALPH( 2) = (   -51.9023,    45.7527)

ALPH( 4) - ALPH( 1) = (   -69.3843,    29.4838)
ALPH( 4) - ALPH( 2) = (   -66.9053,    31.9624)
ALPH( 4) - ALPH( 3) = (   -63.8310,    35.0371)

ALPH( 5) - ALPH( 1) = (  -119.6744,   -19.4825)
ALPH( 5) - ALPH( 2) = (  -117.1958,   -17.0039)
ALPH( 5) - ALPH( 3) = (  -114.1211,   -13.9292)
ALPH( 5) - ALPH( 4) = (  -100.3155,     1.0592)

ALPH( 6) - ALPH( 1) = (  -111.6213,   -13.9653)
ALPH( 6) - ALPH( 2) = (  -109.1427,   -11.4877)
ALPH( 6) - ALPH( 3) = (  -106.0680,    -8.4130)
ALPH( 6) - ALPH( 4) = (   -92.2776,     6.5905)
ALPH( 6) - ALPH( 5) = (   -43.3113,    56.0806)

ALPH( 7) - ALPH( 1) = (  -159.4458,   -59.5777)
ALPH( 7) - ALPH( 2) = (  -155.9673,   -57.0991)
ALPH( 7) - ALPH( 3) = (  -152.8925,   -54.0244)
ALPH( 7) - ALPH( 4) = (  -139.0948,   -39.0282)
ALPH( 7) - ALPH( 5) = (   -90.1207,    11.2540)
ALPH( 7) - ALPH( 6) = (   -95.6520,     3.2161)
```

LDH$_{21}$

```
GROUPE NUMERO  1     MOYENNE=   502.623     ECART-TYPE=   143.750
GROUPE NUMERO  2     MOYENNE=   531.325     ECART-TYPE=   124.157
GROUPE NUMERO  3     MOYENNE=   452.519     ECART-TYPE=   104.753
GROUPE NUMERO  4     MOYENNE=   426.048     ECART-TYPE=   113.817
GROUPE NUMERO  5     MOYENNE=   127.790     ECART-TYPE=    39.352
GROUPE NUMERO  6     MOYENNE=   279.114     ECART-TYPE=    93.921
GROUPE NUMERO  7     MOYENNE=   292.242     ECART-TYPE=    80.129
   MOYENNE GENERALE=      381.162


  SOURCE         SS           DL          MS            F
*************************************************************

 ENTRE LES   .26789E+07       6       .44649E+06    38.241
  GROUPES

  ERREUR     .16813E+07     144       .11675E+05
*************************************************************

  TOTAL      .43602E+07     150
```

```
ALPH( 2) - ALPH( 1) = (   -84.8690,   142.2535)

ALPH( 3) - ALPH( 1) = (  -166.4046,    66.1750)
ALPH( 3) - ALPH( 2) = (  -196.2712,    38.6571)

ALPH( 4) - ALPH( 1) = (  -190.1472,    36.9752)
ALPH( 4) - ALPH( 2) = (  -220.0417,     9.4852)
ALPH( 4) - ALPH( 3) = (  -143.9354,    90.9929)

ALPH( 5) - ALPH( 1) = (  -492.6740,  -257.0127)
ALPH( 5) - ALPH( 2) = (  -522.5254,  -284.5463)
ALPH( 5) - ALPH( 3) = (  -446.3252,  -203.1329)
ALPH( 5) - ALPH( 4) = (  -417.2471,  -179.2685)

ALPH( 6) - ALPH( 1) = (  -339.8094,  -107.2287)
ALPH( 6) - ALPH( 2) = (  -369.6760,  -134.7476)
ALPH( 6) - ALPH( 3) = (  -293.5039,   -53.3006)
ALPH( 6) - ALPH( 4) = (  -264.3977,   -29.4594)
ALPH( 6) - ALPH( 5) = (    29.7231,   272.9204)

ALPH( 7) - ALPH( 1) = (  -329.9013,   -90.8812)
ALPH( 7) - ALPH( 2) = (  -359.7365,  -118.4314)
ALPH( 7) - ALPH( 3) = (  -283.5012,   -37.0527)
ALPH( 7) - ALPH( 4) = (  -254.4583,   -13.1532)
ALPH( 7) - ALPH( 5) = (    39.7732,   289.1310)
ALPH( 7) - ALPH( 6) = (  -110.0964,   136.3521)
```

LDH$_3$

```
GROUPE NUMERO  1    MOYENNE=   412.342    ECART-TYPE=   130.331
GROUPE NUMERO  2    MOYENNE=   448.952    ECART-TYPE=   110.512
GROUPE NUMERO  3    MOYENNE=   384.633    ECART-TYPE=    90.937
GROUPE NUMERO  4    MOYENNE=   366.417    ECART-TYPE=    97.403
GROUPE NUMERO  5    MOYENNE=   137.010    ECART-TYPE=    41.839
GROUPE NUMERO  6    MOYENNE=   327.914    ECART-TYPE=   103.537
GROUPE NUMERO  7    MOYENNE=   249.574    ECART-TYPE=    30.943
   MOYENNE GENERALE=    330.379


   SOURCE          SS            DL         MS             F
***************************************************************

   ENTRE LES    .14385E+07       6      .23977E+06     24.306
   GROUPES

   ERREUR       .14205E+07     144      .98649E+04
***************************************************************

   TOTAL        .28592E+07     150
```

```
ALPH( 2) - ALPH( 1) = (   -67.7745,   140.9955)


ALPH( 3) - ALPH( 1) = (  -134.6014,    79.1847)
ALPH( 3) - ALPH( 2) = (  -172.2909,    43.6532)


ALPH( 4) - ALPH( 1) = (  -150.3092,    58.4607)
ALPH( 4) - ALPH( 2) = (  -180.0244,    22.9548)
ALPH( 4) - ALPH( 3) = (  -126.1030,    89.7561)


ALPH( 5) - ALPH( 1) = (  -303.6406,  -167.0227)
ALPH( 5) - ALPH( 2) = (  -421.3161,  -202.5582)
ALPH( 5) - ALPH( 3) = (  -359.3935,  -135.3532)
ALPH( 5) - ALPH( 4) = (  -338.7813,  -120.0334)


ALPH( 6) - ALPH( 1) = (  -191.3205,    22.4557)
ALPH( 6) - ALPH( 2) = (  -229.0099,   -13.0558)
ALPH( 6) - ALPH( 3) = (  -167.1177,    53.6795)
ALPH( 6) - ALPH( 4) = (  -146.4752,    69.4689)
ALPH( 6) - ALPH( 5) = (    79.1341,   302.6744)


ALPH( 7) - ALPH( 1) = (  -272.6206,   -52.9154)
ALPH( 7) - ALPH( 2) = (  -310.2813,   -88.4757)
ALPH( 7) - ALPH( 3) = (  -243.3253,   -21.7930)
ALPH( 7) - ALPH( 4) = (  -227.7465,    -5.9409)
ALPH( 7) - ALPH( 5) = (    -2.0401,   227.1675)
ALPH( 7) - ALPH( 6) = (  -191.6073,    34.9251)
```

LDH_{21}/LDH_3

```
GROUPE NUMERO  1     MOYENNE=      1.229      ECART-TYPE=       .101
GROUPE NUMERO  2     MOYENNE=      1.193      ECART-TYPE=       .097
GROUPE NUMERO  3     MOYENNE=      1.184      ECART-TYPE=       .116
GROUPE NUMERO  4     MOYENNE=      1.165      ECART-TYPE=       .099
GROUPE NUMERO  5     MOYENNE=       .942      ECART-TYPE=       .113
GROUPE NUMERO  6     MOYENNE=       .851      ECART-TYPE=       .070
GROUPE NUMERO  7     MOYENNE=      1.189      ECART-TYPE=       .083
   MOYENNE GENERALE=       1.113



  SOURCE        SS           DL          MS            F
*************************************************************

  ENTRE LES   .26603E+01      5      .44489E+00    45.094
   GROUPES

  ERREUR      .13899E+01     144     .95510E-02
*************************************************************

  TOTAL       .40592E+01     150
```

```
ALPH( 2) - ALPH( 1) = (     -.1309,      .0675)


ALPH( 3) - ALPH( 1) = (     -.1511,      .0604)
ALPH( 3) - ALPH( 2) = (     -.1165,      .0971)


ALPH( 4) - ALPH( 1) = (     -.1672,      .0393)
ALPH( 4) - ALPH( 2) = (     -.1326,      .0761)
ALPH( 4) - ALPH( 3) = (     -.1254,      .0882)


ALPH( 5) - ALPH( 1) = (     -.3943,     -.1800)
ALPH( 5) - ALPH( 2) = (     -.3597,     -.1433)
ALPH( 5) - ALPH( 3) = (     -.3524,     -.1313)
ALPH( 5) - ALPH( 4) = (     -.3314,     -.1150)


ALPH( 6) - ALPH( 1) = (     -.4739,     -.2525)
ALPH( 6) - ALPH( 2) = (     -.4393,     -.2257)
ALPH( 6) - ALPH( 3) = (     -.4321,     -.2137)
ALPH( 6) - ALPH( 4) = (     -.4111,     -.1975)
ALPH( 6) - ALPH( 5) = (     -.1916,      .0295)


ALPH( 7) - ALPH( 1) = (     -.1409,      .0584)
ALPH( 7) - ALPH( 2) = (     -.1142,      .1052)
ALPH( 7) - ALPH( 3) = (     -.1069,      .1172)
ALPH( 7) - ALPH( 4) = (     -.0860,      .1334)
ALPH( 7) - ALPH( 5) = (      .1336,      .3503)
ALPH( 7) - ALPH( 6) = (      .2160,      .4400)
```

M - LDH

```
GROUPE NUMERO  1     MOYENNE=   449.892     ECART-TYPE=   144.429
GROUPE NUMERO  2     MOYENNE=   443.339     ECART-TYPE=   125.175
GROUPE NUMERO  3     MOYENNE=   377.110     ECART-TYPE=   120.733
GROUPE NUMERO  4     MOYENNE=   345.852     ECART-TYPE=   130.395
GROUPE NUMERO  5     MOYENNE=    46.110     ECART-TYPE=    47.760
GROUPE NUMERO  6     MOYENNE=    43.700     ECART-TYPE=    56.970
GROUPE NUMERO  7     MOYENNE=   241.111     ECART-TYPE=    54.560
   MOYENNE GENERALE=     287.444
```

```
   SOURCE         SS           DL         MS          F
*************************************************************

  ENTRE LES   .39293E+07      6      .65439E+06    55.455
   GROUPES

  ERREUR      .17002E+07     144     .11807E+05
*************************************************************

   TOTAL      .56295E+07     150
```

```
ALPH( 2) - ALPH( 1) = (  -115.7516,   112.6465)

ALPH( 3) - ALPH( 1) = (  -189.7251,    44.1608)
ALPH( 3) - ALPH( 2) = (  -189.3580,    45.8930)

ALPH( 4) - ALPH( 1) = (  -218.2385,    10.1596)
ALPH( 4) - ALPH( 2) = (  -217.8945,    12.9206)
ALPH( 4) - ALPH( 3) = (  -149.3007,    65.0660)

ALPH( 5) - ALPH( 1) = (  -522.2736,  -285.2397)
ALPH( 5) - ALPH( 2) = (  -521.8862,  -282.5721)
ALPH( 5) - ALPH( 3) = (  -453.2781,  -209 7210)
ALPH( 5) - ALPH( 4) = (  -419.3993,  -160.0951)

ALPH( 6) - ALPH( 1) = (  -523.1346,  -289.2407)
ALPH( 6) - ALPH( 2) = (  -522.7625,  -285.5158)
ALPH( 6) - ALPH( 3) = (  -454.1877,  -212.6314)
ALPH( 6) - ALPH( 4) = (  -420.2755,  -184.0283)
ALPH( 6) - ALPH( 5) = (  -124.6886,   119.0535)

ALPH( 7) - ALPH( 1) = (  -328.9619,   -88.6004)
ALPH( 7) - ALPH( 2) = (  -328.5533,   -85.8939)
ALPH( 7) - ALPH( 3) = (  -259.9143,   -12.0332)
ALPH( 7) - ALPH( 4) = (  -226.0713,    16.5030)
ALPH( 7) - ALPH( 5) = (    69.6519,   320.3792)
ALPH( 7) - ALPH( 6) = (    73.4947,   321.3263)
```

```
H - LDH


GROUPE NUMERO  1      MOYENNE=    65.937      ECART-TYPE=     94.093
GROUPE NUMERO  2      MOYENNE=   103.727      ECART-TYPE=     92.131
GROUPE NUMERO  3      MOYENNE=    94.257      ECART-TYPE=     99.957
GROUPE NUMERO  4      MOYENNE=    98.065      ECART-TYPE=     77.980
GROUPE NUMERO  5      MOYENNE=    99.930      ECART-TYPE=     50.379
GROUPE NUMERO  6      MOYENNE=   291.875      ECART-TYPE=    114.579
GROUPE NUMERO  7      MOYENNE=    61.297      ECART-TYPE=     61.835
   MOYENNE GENERALE=     115.866


  SOURCE        SS          DL          MS           F
*****************************************************************

  ENTRE LES   .79254E+06     6       .13209E+06    17.227
   GROUPES

  ERREUR      .11041E+07    144      .75576E+04
*****************************************************************

  TOTAL       .18967E+07    150
```

```
ALPH( 2) - ALPH( 1) = (   -54.2386,   129.8184)

ALPH( 3) - ALPH( 1) = (   -65.9200,   122.5593)
ALPH( 3) - ALPH( 2) = (  -104.6612,    85.7207)

ALPH( 4) - ALPH( 1) = (   -59.9007,   124.1562)
ALPH( 4) - ALPH( 2) = (   -98.6645,    87.3402)
ALPH( 4) - ALPH( 3) = (   -91.3829,    98.9990)

ALPH( 5) - ALPH( 1) = (   -61.4954,   129.4804)
ALPH( 5) - ALPH( 2) = (  -100.2243,    92.6295)
ALPH( 5) - ALPH( 3) = (   -92.8666,   104.2123)
ALPH( 5) - ALPH( 4) = (   -94.5621,    98.2917)

ALPH( 6) - ALPH( 1) = (   131.6990,   320.1784)
ALPH( 6) - ALPH( 2) = (    92.9579,   283.3397)
ALPH( 6) - ALPH( 3) = (   100.2887,   294.9494)
ALPH( 6) - ALPH( 4) = (    98.6200,   289.0019)
ALPH( 6) - ALPH( 5) = (    93.4060,   290.4856)

ALPH( 7) - ALPH( 1) = (  -101.4890,    92.2087)
ALPH( 7) - ALPH( 2) = (  -140.2048,    55.3447)
ALPH( 7) - ALPH( 3) = (  -132.8186,    66.8990)
ALPH( 7) - ALPH( 4) = (  -134.5425,    61.0059)
ALPH( 7) - ALPH( 5) = (  -139.6703,    62.4050)
ALPH( 7) - ALPH( 6) = (  -330.4375,  -130.7200)
```

TOTAL LDH

```
GROUPE NUMERO  1    MOYENNE=   513.321      ECART-TYPE=   155.745
GROUPE NUMERO  2    MOYENNE=   541.200      ECART-TYPE=   127.214
GROUPE NUMERO  3    MOYENNE=   471.371      ECART-TYPE=   107.552
GROUPE NUMERO  4    MOYENNE=   440.104      ECART-TYPE=   124.450
GROUPE NUMERO  5    MOYENNE=   143.595      ECART-TYPE=    41.200
GROUPE NUMERO  6    MOYENNE=   342.729      ECART-TYPE=   169.141
GROUPE NUMERO  7    MOYENNE=   305.032      ECART-TYPE=    89.716
   MOYENNE GENERALE=     401.756



  SOURCE        SS          DL        MS          F
*************************************************************

 ENTRE LES   .24602E+07      6      .41137E+06    31.035
 GROUPES

 ERREUR      .19087E+07    144      .13255E+05
*************************************************************

  TOTAL      .43769E+07    150
```

```
ALPH( 2) - ALPH( 1) = (   -93.6182,   148.3766)

ALPH( 3) - ALPH( 1) = (  -166.3541,    81.4553)
ALPH( 3) - ALPH( 2) = (  -194.9339,    55.3268)

ALPH( 4) - ALPH( 1) = (  -194.7139,    47.2809)
ALPH( 4) - ALPH( 2) = (  -223.3735,    21.1822)
ALPH( 4) - ALPH( 3) = (  -156.4224,    93.8833)

ALPH( 5) - ALPH( 1) = (  -495.7817,  -244.6900)
ALPH( 5) - ALPH( 2) = (  -524.3954,  -270.8346)
ALPH( 5) - ALPH( 3) = (  -457.3444,  -198.2235)
ALPH( 5) - ALPH( 4) = (  -423.2997,  -169.7390)

ALPH( 6) - ALPH( 1) = (  -294.9969,   -47.1876)
ALPH( 6) - ALPH( 2) = (  -323.6268,   -73.3161)
ALPH( 6) - ALPH( 3) = (  -256.6111,     -.6747)
ALPH( 6) - ALPH( 4) = (  -222.5311,    27.7795)
ALPH( 6) - ALPH( 5) = (    69.5056,   328.7015)

ALPH( 7) - ALPH( 1) = (  -336.1245,   -81.4540)
ALPH( 7) - ALPH( 2) = (  -364.7209,  -107.6159)
ALPH( 7) - ALPH( 3) = (  -297.6324,   -35.0473)
ALPH( 7) - ALPH( 4) = (  -263.6253,    -6.5202)
ALPH( 7) - ALPH( 5) = (    28.6040,   294.2331)
ALPH( 7) - ALPH( 6) = (  -168.8896,    93.5956)
```

APPENDIX F

TRAINING GROUP AND ORGAN COMPARISONS
FOR EACH DEPENDANT VARIABLE:
TWO WAY ANALYSES OF VARIANCE
AND OTHER RELATED STATISTICS

This appendix contains the two by two factors analysis of variance in the usual order:

1. W_{abs}
2. W_{rel}
3. ATP
4. PC
5. ATP + PC
6. CPK
7. AK
8. LDH_{21}
9. LDH_3
10. LDH_{21}/LDH_3
11. M-LDH
12. M-LDH
13. TOTAL LDH

```
                                        W ABS                                    30/03/77        PAGE
MUSCLE VS ANIMAL (2).
FILE    NONAME    (CREATION DATE =  30/03/77 )
* * * * * * * * * * A N A L Y S I S   O F   V A R I A N C E * * * * * * * * * * * *
                  MESURE
               BY MUSCLE
                  ANIMAL
* * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * *

                                      SUM OF                   MEAN              SIGNIF
SOURCE OF VARIATION                  SQUARES         DF       SQUARE        F      OF F

MAIN EFFECTS                     3562646211.830       8  445330776.479  320.656    .001
   MUSCLE                        3555524435.251       6  592587405.875  426.687    .001
   ANIMAL                           7744340.488       2    3872170.244    2.788    .065

2-WAY INTERACTIONS                 44645100.319      12    3720425.027    2.679    .003
   MUSCLE    ANIMAL                44645100.319      12    3720425.027    2.679    .003

EXPLAINED                        3607291312.149      20  180364565.607  129.870    .001

RESIDUAL                          195822410.584     141    1388811.451

TOTAL                            3803113726.733     161   23621824.390

   168 CASES WERE PROCESSED.
     6 CASES (  3.6 PCT) WERE MISSING.

CODE     VALUE LABEL                SUM          MEAN        STD DEV       VARIANCE        N

                                 .473E+06     2924.7169     4860.2288     .236E+08    (   162)

   1                           23751.5000      989.6458       88.8807     7894.2661    (    24)
   2                           13552.0000      564.6667       57.2177     3273.8632    (    24)
   3                           29278.9000     1219.9542       99.8135     9962.7339    (    24)
   4                           34362.1000     1431.7542      151.5706    22973.6417    (    24)
   5                            5828.9000      253.4304       35.4477     1256.5395    (    23)
   6                           28293.3000     1489.1211      132.3294    17511.0662    (    19)
   7                             .338E+06    14114.0600     3276.0966     .107E+08    (    24)

CODE     VALUE LABEL                SUM          MEAN        STD DEV       VARIANCE        N

                                 .473E+06     2924.7169     4860.2288     .236E+08    (   162)

   1                             .144E+06     2683.0063     4470.5066     .199E+08    (    54)
   2                             .156E+06     2896.8296     4707.8041     .221E+08    (    54)
   3                             .172E+06     3194.3148     5428.9632     .294E+08    (    54)
```

W_{rel}

```
MUSCLE VS ANIMAL (3).                                                                    30/03/77        PAGE
FILE    NONAME    (CREATION DATE =  30/03/77 )
* * * * * * * * * * * A N A L Y S I S   O F   V A R I A N C E * * * * * * * * * * *
                  MESURE
              BY  MUSCLE
                  ANIMAL
* * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * *

                                          SUM OF                    MEAN                SIGNIF
SOURCE OF VARIATION                      SQUARES       DF          SQUARE        F       OF F
MAIN EFFECTS                           12112.719        8        1514.090  862.737      .001
   MUSCLE                              12110.342        6        2018.390 1150.090      .001
   ANIMAL                                  2.038        2           1.019     .581      .561
2-WAY INTERACTIONS                          .867       12            .072     .041      .999
   MUSCLE    ANIMAL                         .867       12            .072     .041      .999
EXPLAINED                              12113.586       20         605.679  345.120      .001
RESIDUAL                                 247.453      141           1.755
TOTAL                                  12361.039      161          76.777

    168 CASES WERE PROCESSED.
      6 CASES (  3.6 PCT) WERE MISSING.

 CODE    VALUE LABEL               SUM          MEAN        STD DEV      VARIANCE        N
                                865.3960       5.3420        8.7622       76.7766     (  162)
  1                              42.5870       1.7745         .2320         .0538     (   24)
  2                              24.3320       1.0138         .1471         .0216     (   24)
  3                              52.4790       2.1866         .2654         .0705     (   24)
  4                              61.6150       2.5673         .3712         .1378     (   24)
  5                              10.4250        .4533         .0754         .0057     (   23)
  6                              49.9510       2.6290         .3359         .1128     (   19)
  7                             624.0070      26.0003        3.2416       10.5077     (   24)
 CODE    VALUE LABEL               SUM          MEAN        STD DEV      VARIANCE        N
                                865.3960       5.3420        8.7622       76.7766     (  162)
  1                             293.4000       5.4333        8.7563       76.6736     (   54)
  2                             292.7740       5.4217        8.7809       77.1040     (   54)
  3                             279.2220       5.1708        8.9109       79.4047     (   54)
```

ATP

```
MUSCLE VS ANIMAL (4).                                                                              30/03/77        PAGE
FILE    NONAME    (CREATION DATE =  30/03/77 )
* * * * * * * * * * * A N A L Y S I S   O F   V A R I A N C E * * * * * * * * * * *
                       MESURE
                 BY MUSCLE
                       ANIMAL
* * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * *

                                         SUM OF                     MEAN               SIGNIF
SOURCE OF VARIATION                      SQUARES        DF         SQUARE       F       OF F

MAIN EFFECTS                              79.887         7         11.412   26.114      .001
   MUSCLE                                 63.747         5         12.749   29.173      .001
   ANIMAL                                 16.006         2          8.003   18.312      .001

2-WAY INTERACTIONS                         5.159        10           .516    1.180      .310
   MUSCLE    ANIMAL                        5.159        10           .516    1.180      .310

EXPLAINED                                 85.046        17          5.003   11.447      .001

RESIDUAL                                  53.755       123           .437

TOTAL                                    138.801       140           .991

    144 CASES WERE PROCESSED.
      3 CASES (   2.1 PCT) WERE MISSING.

 CODE     VALUE LABEL                    SUM            MEAN         STD DEV       VARIANCE         N

                                      661.7600         4.6933          .9957          .9914    (    141)

   1                                  131.9400         5.4975          .7653          .5857    (     24)
   2                                  114.6500         4.9848          .7130          .5084    (     23)
   3                                  123.9000         5.1625         1.0712         1.1474    (     24)
   4                                  114.9200         4.7883          .7642          .5840    (     24)
   5                                   83.1000         3.4625          .5220          .2725    (     24)
   6                                   93.2500         4.2386          .4459          .1988    (     22)

 CODE     VALUE LABEL                    SUM            MEAN         STD DEV       VARIANCE         N

                                      661.7600         4.6933          .9957          .9914    (    141)

   1                                  205.3400         4.2779          .7219          .5211    (     48)
   2                                  234.8800         5.1061         1.0400         1.0816    (     46)
   3                                  221.5400         4.7136         1.0373         1.0760    (     47)
```

PC

```
MUSCLE VS ANIMAL (5).                                                                  30/03/77         PAGE
FILE   NONAME   (CREATION DATE =  30/03/77 )
* * * * * * * * * * * A N A L Y S I S   O F   V A R I A N C E * * * * * * * * * * *
                MESURE
             BY MUSCLE
                ANIMAL
* * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * *

SOURCE OF VARIATION               SUM OF                  MEAN              SIGNIF
                                  SQUARES      DF         SQUARE      F      OF F
MAIN EFFECTS                     2211.308       7        315.901   33.000    .001
   MUSCLE                        2200.249       5        440.050   45.969    .001
   ANIMAL                           9.342       2          4.671     .488    .615
2-WAY INTERACTIONS                 59.744      10          5.974     .624    .790
   MUSCLE    ANIMAL                59.744      10          5.974     .624    .790
EXPLAINED                        2271.053      17        133.591   13.955    .001
RESIDUAL                          938.138      98          9.573
TOTAL                            3209.190     115         27.906

    144 CASES WERE PROCESSED.
     28 CASES ( 19.4 PCT) WERE MISSING.

CODE    VALUE LABEL                SUM          MEAN        STD DEV      VARIANCE        N
                                1525.5000      13.1509       5.2826       27.9060   (   116)
  1                              366.1700      18.3085       3.0309        9.1862   (    20)
  2                              294.7100      14.7355       4.5711       20.8948   (    20)
  3                              326.2100      16.3105       3.1101        9.6725   (    20)
  4                              269.9500      14.2079       2.6738        7.1494   (    19)
  5                              170.1700       8.9563       2.2584        5.1065   (    19)
  6                               98.2900       5.4606       1.3593        1.8477   (    18)
CODE    VALUE LABEL                SUM          MEAN        STD DEV      VARIANCE        N
                                1525.5000      13.1509       5.2826       27.9060   (   116)
  1                              443.8400      12.6811       5.1380       26.3990   (    35)
  2                              547.4100      13.3515       4.9499       24.5020   (    41)
  3                              534.2500      13.3563       5.8188       33.8586   (    40)
```

ATP & PC

```
MUSCLE VS ANIMAL (6).                                                          30/03/77       PAGE
FILE    NONAME    (CREATION DATE =  30/03/77 )
* * * * * * * * * * A N A L Y S I S   O F   V A R I A N C E * * * * * * * * * * *
                    MESURE
             BY MUSCLE
                    ANIMAL
* * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * *

                                        SUM OF               MEAN             SIGNIF
SOURCE OF VARIATION                    SQUARES     DF       SQUARE       F      OF F

MAIN EFFECTS                          2926.672      7      418.096   38.741    .001
   MUSCLE                             2893.747      5      578.749   53.627    .001
   ANIMAL                               37.949      2       18.975    1.758    .178

2-WAY INTERACTIONS                      90.389     10        9.039     .838    .594
   MUSCLE    ANIMAL                     90.389     10        9.039     .838    .594

EXPLAINED                             3017.061     17      177.474   16.445    .001

RESIDUAL                              1068.416     99       10.792

TOTAL                                 4085.477    116       35.220

    144 CASES WERE PROCESSED.
     27 CASES ( 18.7 PCT) WERE MISSING.

CODE    VALUE LABEL             SUM          MEAN        STD DEV      VARIANCE        N

                             2079.3300      17.7721       5.9346      35.2196    (  117)

  1                           474.7100      23.7355       3.0938       9.5718    (   20)
  2                           393.9600      19.6980       4.6843      21.9428    (   20)
  3                           429.4100      21.4705       3.7232      13.8623    (   20)
  4                           361.9800      19.0516       3.0276       9.1661    (   19)
  5                           235.9800      12.4200       2.5950       6.7338    (   19)
  6                           183.2900       9.6468       1.6397       2.6886    (   19)

CODE    VALUE LABEL             SUM          MEAN        STD DEV      VARIANCE        N

                             2079.3300      17.7721       5.9346      35.2196    (  117)

  1                           593.6300      16.9609       5.5890      31.2370    (   35)
  2                           762.1100      18.1455       5.8000      33.6404    (   42)
  3                           723.5900      18.0898       6.4276      41.3138    (   40)
```

CPK

```
MUSCLE VS ANIMAL (7).                                                                       30/03/77       PAGE
FILE    NONAME   (CREATION DATE =  30/03/77 )
* * * * * * * * * * * A N A L Y S I S   O F   V A R I A N C E * * * * * * * * * * * *
                    MESURE
                 BY MUSCLE
                    ANIMAL
* * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * *

                                       SUM OF                    MEAN               SIGNIF
SOURCE OF VARIATION                   SQUARES       DF          SQUARE        F       OF F

MAIN EFFECTS                      148203592.524      8    18525449.066  175.594     .001
   MUSCLE                         144482879.882      6    24080479.980  228.248     .001
   ANIMAL                           3448123.851      2     1724061.926   16.342     .001

2-WAY INTERACTIONS                  4344881.810     12      362073.484    3.432     .001
   MUSCLE    ANIMAL                 4344881.810     12      362073.484    3.432     .001

EXPLAINED                         152548474.335     20     7627423.717   72.297     .001

RESIDUAL                           14770218.208    140      105501.559

TOTAL                             167318692.543    160     1045741.828

    168 CASES WERE PROCESSED.
      7 CASES (  4.2 PCT) WERE MISSING.

CODE     VALUE LABEL                     SUM            MEAN          STD DEV          VARIANCE        N

                                     .269E+06       1673.5701       1022.6152         .104E+07    (    161)

  1                                54375.7000       2589.3190        405.8792         .164E+06    (     21)
  2                                57188.2000       2486.4435        452.7908         .205E+06    (     23)
  3                                58852.4000       2558.8000        541.6018         .293E+06    (     23)
  4                                53049.7000       2210.4042        557.1782         .310E+06    (     24)
  5                                24765.0000       1076.7391        166.0117       27559.8852    (     23)
  6                                20886.0000        870.2500        155.5426       24193.4896    (     24)
  7                                  327.7900         14.2517          8.6878          75.4781    (     23)
CODE     VALUE LABEL                     SUM            MEAN          STD DEV          VARIANCE        N

                                     .269E+06       1673.5701       1022.6152         .104E+07    (    161)

  1                                86397.5200       1570.8640        901.8166         .813E+06    (     55)
  2                                82849.1300       1563.1911        947.2194         .897E+06    (     53)
  3                                  .100E+06       1890.5309       1185.0475         .140E+07    (     53)
```

AK

```
MUSCLE VS ANIMAL (8).                                                                    30/03/77      PAGE
FILE   NONAME   (CREATION DATE =  30/03/77 )
* * * * * * * * * * * A N A L Y S I S   O F   V A R I A N C E * * * * * * * * * * *
            MESURE
         BY MUSCLE
            ANIMAL
* * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * *

                                       SUM OF                MEAN              SIGNIF
SOURCE OF VARIATION                    SQUARES      DF      SQUARE       F       OF F
MAIN EFFECTS                         219719.009      8    27464.876   12.967     .001
   MUSCLE                            218602.811      6    36433.802   17.202     .001
   ANIMAL                              1578.294      2      789.147     .373     .690

2-WAY INTERACTIONS                     2000.367     12      166.697     .079     .999
   MUSCLE   ANIMAL                     2000.367     12      166.697     .079     .999

EXPLAINED                            221719.376     20    11085.969    5.234     .001

RESIDUAL                             258403.028    122     2118.058

TOTAL                                480122.404    142     3381.144

    168 CASES WERE PROCESSED.
     25 CASES ( 14.9 PCT) WERE MISSING.

CODE    VALUE LABEL              SUM          MEAN        STD DEV      VARIANCE        N

                             12984.7500      90.8024      58.1476     3381.1437   (   143)

  1                           2698.1800     128.4848      55.5698     3088.0009   (    21)
  2                           2646.1300     126.0062      57.0023     3253.8205   (    21)
  3                           2581.5600     122.9314      56.4076     3181.8216   (    21)
  4                           2170.6900     108.5345      49.6006     2460.2195   (    20)
  5                           1119.2200      58.9063      25.7786      664.5387   (    19)
  6                           1379.5100      65.6910      24.9104      620.5301   (    21)
  7                            389.4600      19.4730       4.5443       20.6505   (    20)
CODE    VALUE LABEL              SUM          MEAN        STD DEV      VARIANCE        N

                             12984.7500      90.8024      58.1476     3381.1437   (   143)

  1                           4499.2300      93.7340      50.3266     2532.7685   (    48)
  2                           4626.3000      87.2887      65.0227     4227.9457   (    53)
  3                           3859.2200      91.8862      58.4584     3417.3981   (    42)
```

LDH_{21}

```
MUSCLE VS ANIMAL (9).                                                              30/03/77        PAGE
FILE  NONAME   (CREATION DATE =  30/03/77 )
* * * * * * * * * * A N A L Y S I S   O F   V A R I A N C E * * * * * * * * * * *
                 MESURE
            BY MUSCLE
                 ANIMAL
* * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * *

                                      SUM OF                   MEAN            SIGNIF
SOURCE OF VARIATION                   SQUARES       DF        SQUARE        F   OF F

MAIN EFFECTS                       2831604.179       8    353950.522   32.105   .001
   MUSCLE                          2738086.846       6    456347.808   41.393   .001
   ANIMAL                           152713.101       2     76356.551    6.926   .001

2-WAY INTERACTIONS                   95359.430      12      7946.619     .721   .729
   MUSCLE    ANIMAL                  95359.430      12      7946.619     .721   .729

EXPLAINED                          2926963.608      20    146348.180   13.275   .001

RESIDUAL                           1433216.447     130     11024.742

TOTAL                              4360180.055     150     29067.867

    168 CASES WERE PROCESSED.
     17 CASES ( 10.1 PCT) WERE MISSING.

 CODE    VALUE LABEL                SUM          MEAN        STD DEV      VARIANCE        N

                                57555.5000     381.1623     170.4930    29067.8670   (   151)

   1                            12063.2000     502.6333     148.7502    22126.6223   (    24)
   2                            12220.5000     531.3261     124.1666    15417.3429   (    23)
   3                             9502.9000     452.5190     104.7530    10973.1936   (    21)
   4                             9799.1000     426.0478     118.8168    14117.0326   (    23)
   5                             2555.8000     127.7900      39.3620     1549.3641   (    20)
   6                             5861.4000     279.1143      88.9210     7906.9453   (    21)
   7                             5552.6000     292.2421      80.1267     6420.6059   (    19)
 CODE    VALUE LABEL                SUM          MEAN        STD DEV      VARIANCE        N

                                57555.5000     381.1623     170.4930    29067.8670   (   151)

   1                            18509.8000     362.9373     156.2590    24416.8816   (    51)
   2                            18219.6000     364.3920     156.6302    24533.0276   (    50)
   3                            20826.1000   ->416.5220     193.4556    37424.5405   (    50)
```

LDH$_3$

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MUSCLE VS ANIMAL (10).                                                         30/03/77      PAGE
FILE    NONAME    (CREATION DATE =  30/03/77 )
* * * * * * * * * * A N A L Y S I S   O F   V A R I A N C E * * * * * * * * * * *
                  MESURE
              BY MUSCLE
                  ANIMAL
* * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * *

                               SUM OF                     MEAN               SIGNIF
SOURCE OF VARIATION           SQUARES        DF          SQUARE        F      OF F

MAIN EFFECTS                1571238.100       8      196404.763   20.897     .001
   MUSCLE                   1493238.227       6      248873.038   26.479     .001
   ANIMAL                    132598.150       2       66299.075    7.054     .001

2-WAY INTERACTIONS            66097.129      12        5508.094     .586     .850
   MUSCLE    ANIMAL           66097.129      12        5508.094     .586     .850

EXPLAINED                   1637335.229      20       81866.761    8.710     .001

RESIDUAL                    1221847.537     130        9398.827

TOTAL                       2859182.766     150       19061.218

     168 CASES WERE PROCESSED.
      17 CASES ( 10.1 PCT) WERE MISSING.

 CODE    VALUE LABEL              SUM          MEAN        STD DEV       VARIANCE         N

                             51095.3000      338.3795     138.0624     19061.2184    (   151)

   1                          9896.2000      412.3417     130.3306     16986.0756    (    24)
   2                         10325.9000      448.9522     110.5116     12212.8035    (    23)
   3                          8077.3000      384.6333      90.9370      8269.5413    (    21)
   4                          8427.6000      366.4174      97.4052      9487.7633    (    23)
   5                          2740.2000      137.0100      41.8593      1752.2009    (    20)
   6                          6886.2000      327.9143     108.5869     11791.1123    (    21)
   7                          4741.9000      249.5737      80.9478      6552.5398    (    19)
 CODE    VALUE LABEL              SUM          MEAN        STD DEV       VARIANCE         N

                             51095.3000      338.3795     138.0624     19061.2184    (   151)

   1                         16718.1000      327.8059     127.3941     16229.2550    (    51)
   2                         15871.5000      317.4300     131.0128     17164.3479    (    50)
   3                         18505.7000      370.1140     151.7696     23034.0225    (    50)
```

LDH_{21}/LDH_3

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MUSCLE VS ANIMAL (11).                                                                  30/03/77       PAGE

FILE    NONAME   (CREATION DATE =  30/03/77 )

* * * * * * * * * A N A L Y S I S   O F   V A R I A N C E * * * * * * * * * * * *
                  MESURE
              BY  MUSCLE
                  ANIMAL

* * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * *

                                         SUM OF                      MEAN              SIGNIF
SOURCE OF VARIATION                      SQUARES        DF           SQUARE       F     OF F

MAIN EFFECTS                              2.711          8            .339   34.009     .001
   MUSCLE                                 2.640          6            .440   44.728     .001
   ANIMAL                                  .041          2            .021    2.105     .126

2-WAY INTERACTIONS                         .070         12            .006     .591     .846
   MUSCLE    ANIMAL                        .070         12            .006     .591     .846

EXPLAINED                                 2.781         20            .139   14.154     .001

RESIDUAL                                  1.279        130            .010

TOTAL                                     4.059        150            .027

     168 CASES WERE PROCESSED.
      17 CASES ( 10.1 PCT) WERE MISSING.

  CODE    VALUE LABEL                     SUM           MEAN          STD DEV       VARIANCE        N

                                       168.1200        1.1134          .1645         .0271     (   151)

   1                                    29.5000        1.2292          .1011         .0102     (    24)
   2                                    27.4500        1.1935          .0966         .0093     (    23)
   3                                    24.8600        1.1838          .1162         .0135     (    21)
   4                                    26.8000        1.1652          .0988         .0098     (    23)
   5                                    18.8400         .9420          .1133         .0128     (    20)
   6                                    18.0800         .8610          .0701         .0049     (    21)
   7                                    22.5900        1.1889          .0827         .0068     (    19)

  CODE    VALUE LABEL                     SUM           MEAN          STD DEV       VARIANCE        N

                                       168.1200        1.1134          .1645         .0271     (   151)

   1                                    55.9600        1.0973          .1690         .0286     (    51)
   2                                    57.2100        1.1442          .1629         .0265     (    50)
   3                                    54.9500        1.0990          .1604         .0257     (    50)
```

M - LDH

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MUSCLE VS ANIMAL (13).                                                                      30/03/77        PAGE
FILE    NONAME    (CREATION DATE =  30/03/77 )
* * * * * * * * * * A N A L Y S I S   O F   V A R I A N C E * * * * * * * * * * *
               MESURE
            BY MUSCLE
               ANIMAL
* * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * *

                                       SUM OF                 MEAN              SIGNIF
SOURCE OF VARIATION                   SQUARES      DF        SQUARE        F     OF F

MAIN EFFECTS                       4006725.394      8     500840.674   43.098    .001
   MUSCLE                          3940315.466      6     656719.244   56.506    .001
   ANIMAL                            77409.165      2      38704.583    3.330    .039

2-WAY INTERACTIONS                  111937.884     12       9328.157     .803    .647
   MUSCLE    ANIMAL                 111937.884     12       9328.157     .803    .647

EXPLAINED                          4118663.278     20     205933.164   17.719    .001

RESIDUAL                           1510866.055    130      11622.047

TOTAL                              5629529.333    150      37530.196

    168 CASES WERE PROCESSED.
     17 CASES ( 10.1 PCT) WERE MISSING.

 CODE      VALUE LABEL              SUM           MEAN         STD DEV       VARIANCE        N

                               43404.1000       287.4444      193.7271     37530.1956   (   151)

   1                           10797.4000       449.8917      144.4295     20859.8756   (    24)
   2                           10311.8000       448.3391      126.1764     15920.4734   (    23)
   3                            7919.3000       377.1095      128.7379     16573.4359   (    21)
   4                            7954.6000       345.8522      130.8856     17131.0335   (    23)
   5                             922.2000        46.1100       47.7603      2281.0483   (    20)
   6                             917.7000        43.7000       56.9702      3245.6010   (    21)
   7                            4581.1000       241.1105       54.5599      2976.7865   (    19)
 CODE      VALUE LABEL              SUM           MEAN         STD DEV       VARIANCE        N

                               43404.1000       287.4444      193.7271     37530.1956   (   151)

   1                           13572.7000       262.2098      187.9439     35322.9133   (    51)
   2                           14356.7000       287.1340      172.9537     29912.9651   (    50)
   3                           15674.7000       313.4940      218.1199     47576.3940   (    50)
```

```
                                        H - LDH
MUSCLE VS ANIMAL (14).                                                                        30/03/77        PAGE
FILE    NONAME   (CREATION DATE =  30/03/77 )
* * * * * * * * * * A N A L Y S I S   O F   V A R I A N C E * * * * * * * * * * * *
                 MESURE
              BY MUSCLE
                 ANIMAL
* * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * *

                                         SUM OF                      MEAN               SIGNIF
SOURCE OF VARIATION                      SQUARES        DF          SQUARE        F      OF F

MAIN EFFECTS                           830116.723        8       103764.590   13.195     .001
  MUSCLE                               796452.352        6       132742.059   16.880     .001
  ANIMAL                                37578.061        2        18789.031    2.389     .096

2-WAY INTERACTIONS                      44247.114       12         3687.260     .469     .930
  MUSCLE   ANIMAL                       44247.114       12         3687.260     .469     .930

EXPLAINED                              874363.838       20        43718.192    5.559     .001

RESIDUAL                              1022311.292      130         7863.933

TOTAL                                 1896675.130      150        12644.501

     168 CASES WERE PROCESSED.
      17 CASES ( 10.1 PCT) WERE MISSING.

  CODE      VALUE LABEL                 SUM            MEAN          STD DEV       VARIANCE            N

                                   17495.7800        115.8661        112.4478     12644.5009     (    151)

   1                                1582.5000         65.9375         94.0879      8852.5204     (     24)
   2                                2385.7300        103.7274         92.1311      8488.1398     (     23)
   3                                1979.4000         94.2571         99.9573      9991.4625     (     21)
   4                                2255.5000         98.0652         77.9707      6080.8360     (     23)
   5                                1998.6000         99.9300         50.3766      2538.0075     (     20)
   6                                6129.4000        291.8762        114.6787     13151.2009     (     21)
   7                                1164.6500         61.2974         61.8849      3829.7440     (     19)
  CODE      VALUE LABEL                 SUM            MEAN          STD DEV       VARIANCE            N

                                   17495.7800        115.8661        112.4478     12644.5009     (    151)

   1                                6371.0800        124.9231        123.2507     15190.7473     (     51)
   2                                4735.5000         94.7100        104.3069     10879.9393     (     50)
   3                                6389.2000        127.7840        107.8865     11630.0350     (     50)
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TOTAL LDH

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MUSCLE VS ANIMAL (12).                                                                                  30/03/77        PAGE
FILE    NONAME   (CREATION DATE =  30/03/77 )
* * * * * * * * * * * A N A L Y S I S   O F   V A R I A N C E * * * * * * * * * * *
                      MESURE
                   BY MUSCLE
                      ANIMAL
* * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * *

                                            SUM OF                      MEAN                 SIGNIF
SOURCE OF VARIATION                        SQUARES       DF            SQUARE        F        OF F

MAIN EFFECTS                            2643681.550       8        330460.194   26.505      .001
   MUSCLE                               2537002.775       6        422833.796   33.915      .001
   ANIMAL                                175483.319       2         87741.659    7.038      .001

2-WAY INTERACTIONS                       112395.751      12          9366.313     .751      .699
   MUSCLE    ANIMAL                      112395.751      12          9366.313     .751      .699

EXPLAINED                               2756077.301      20        137803.865   11.053      .001

RESIDUAL                                1620789.792     130         12467.614

TOTAL                                   4376867.093     150         29179.114

     168 CASES WERE PROCESSED.
      17 CASES ( 10.1 PCT) WERE MISSING.

  CODE     VALUE LABEL                  SUM            MEAN          STD DEV        VARIANCE          N

                                    60665.1000       401.7556       170.8190      29179.1140     (   151)

    1                               12331.7000       513.8208       156.7451      24569.0460     (    24)
    2                               12447.6000       541.2000       127.2136      16183.3036     (    23)
    3                                9898.8000       471.3714       107.5615      11569.4661     (    21)
    4                               10122.4000       440.1043       124.4598      15490.2350     (    23)
    5                                2871.7000       143.5850        41.1997       1697.4161     (    20)
    6                                7197.3000       342.7286       109.1406      11911.6671     (    21)
    7                                5795.6000       305.0316        89.7184       8049.3956     (    19)
  CODE     VALUE LABEL                  SUM            MEAN          STD DEV        VARIANCE          N

                                    60665.1000       401.7556       170.8190      29179.1140     (   151)

    1                               19497.3000       382.3000       150.4837      22645.3496     (    51)
    2                               19191.7000       383.8340       159.9682      25589.6211     (    50)
    3                               21976.1000       439.5220       196.0851      38449.3797     (    50)
```

APPENDIX G

CHEMICALS USED IN THE STUDY

CHEMICALS	SOURCE	ASSAY(S)
Acrylamide	Fisher, 5521	LDH electrophoresis
ADP, trisodium salt	Sigma A-0127	AK & PC
Adenylate Kinase, grade III, from rabbit muscle in sulfate suspension	Sigma M-3003	AK as a Std
ATP, crystalline disodium salt	Sigma A-3127	ATP as a Std
ATP Assay Kit, Calbiochem ATP - Stat Pack	Calbiochem 869206	ATP
Ammonium persulfate	Fisher A-682	LDH electrophoresis
Bromophenol blue	Fisher B-392	LDH electrophoresis
Creatine Phosphokinase, type 1, from rabbit muscle, lyophilized salt-free powder	Sigma C-3755	PC
CPK Control (Dade)	Canlab B-5126	CPK as a Std
CPK Assays Kit, Dade UV-1-CPK or UV-10-CPK	Canlab B-5329	CPK
Dextrose	Canlab 1916	AK
G-6-P de H, type XV-Baker yeast, crystallized and lyophilized sulfate free	Sigma G-6378	AK
Glycine	Fisher G-46	LDH electrophoresis
Hexokinase, type F-300 sulfate free	Sigma H-4502	AK
Hydrochloric acid	Fisher A-144	LDH electrophoresis
Lactate, Lithium Salt	Sigma L-2250	LDH electrophoresis staining solution
Lactate Dehydrogenase, type II, crystalline from rabbit muscle, ammonium sulfate suspension	Sigma L-2500	LDH and LDH electrophoresis as a Std

CHEMICALS	SOURCE	ASSAY(S)
LDH, type III, from beef heart, ammonium sulfate suspension	Sigma L-2625	LDH and LDH electrophoresis as a Std
LDH, type V, LDH-5 (M_4), isoenzyme, crystalline, rabbit muscle, ammonium sulfate suspension	Sigma L-2875	"
LDH, type VII, LDH-1 (H_4), isoenzyme, crystalline, pig heart, ammonium sulfate suspension	Sigma L-3125	"
Magnesium chloride	Anachemia AC-5538	Ak LDH electrophoresis staining solution
N, N'-Methylenebisacrylamide	Fisher 8383	LDH electrophoresis
Methyl Orange, sodium salt	Canlab 2694 (Baker)	ATP & PC (neutralization)
Nembutal, 50mg/ml	Abbott 3778	Anaesthesia
NAD, free acid	Sigma N-7004	LDH electrophoresis staining solution
NADH, disodium salt, grade III	Sigma N-8129	LDH
NADP, monosodium salt	Sigma N-0505	AK
Nitroblue Tetrazolium	Sigma N-6876	LDH electrophoresis staining solution
Nitrogen (liquid)	University of Montreal (Liquid Air)	ATP & PC (deproteinization)
Perchloric acid, 70% W/V	Canlab 1-9652	ATP & PC (deproteinization)
Phenazine methosulfate	Sigma P-9265	LDH electrophoresis staining solution
Phosphorylcreatine, disodium salt	Sigma P-6502	PC as a Std

CHEMICALS	SOURCE	ASSAY(S)
Potassium acid phosphate	Fisher P-285	LDH buffer, LDH electrophoresis staining solution buffer
Potassium carbonate	University of Montreal 4-5469 (BDH-29591)	ATP & PC (neutralization)
Potassium dichromate	Fisher P-188	AK & CPK (blank)
Pyruvate, sodium salt	Sigma P-2256	LDH
Sodium chloride	Canlab 1-3624 (BDH-10241)	LDH electrophoresis staining solution
Sodium phosphate (dibasic)	Fisher 9-374	LDH buffer LDH electrophoresis staining solution buffer
Sucrose	Canlab 4072	AK, CPK, LDH, LDH electrophoresis (homogenization)
N, N, N', N' - tetramethylethylene diamine	Sigma T-8183	LDH electrophoresis
TRIS (TRIZMA)	Sigma T 1503 (Base) T 3253 (HCL)	AK LDH electrophoresis